CHRONIC TOXICITY SUMMARY

FLUORIDES including HYDROGEN FLUORIDE

hydrofluoric acid (aqueous solution); hydrogen fluoride (as a gas)

CAS Registry Number: 7664-39-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**30 μg HF/m^3; 30 μg F/m^3**
Critical effect(s)	skeletal fluorosis
Hazard index target(s)	Bone; respiratory system

II. Physical and Chemical Properties (HSDB, 1995)

Molecular formula	HF
Molecular weight	20.0
Description	Colorless gas (HF), or as particulates
Specific gravity	0.991 @ 20° C
Boiling point	19.51° C
Vapor pressure	400 mm Hg @ 2.5°C
Solubility	Soluble in water and alcohol
Conversion factor	1 ppm = 0.83 mg/m^3 @ 25° C

III. Major Uses or Sources

Hydrofluoric acid (HF) is a colorless, fuming liquid with a sharp, penetrating odor (Fairhall, 1949). This acid is used in the glass etching, electronic and chemical industries (Bertolini, 1992). These industries use HF in the manufacture of such things as metal cans, plastics, refrigerant chemicals, inorganic chemicals, soaps and detergents, high octane gasoline and aircraft parts (Wohlslagel *et al.*, 1976; Wing *et al.*, 1991).

IV. Effects of Human Exposure

The chronic exposure to fluorides, including HF, and the incidence of osseous changes were studied in the workplace by Derryberry *et al.* (1963). In this study, the 8-hour time-weighted average fluoride exposure was calculated for the employment period of each of 74 workers. The overall average fluoride exposure in these workers was measured as a time-weighted average of

2.81 mg F/m^3. In comparison, the 17 workers within this group who had significantly increased bone density had an average fluoride exposure of 3.38 mg F/m^3. The remainder of the workers were exposed to an average measured concentration of 2.64 mg F/m^3. An analysis of these data by OEHHA (see derivation section below) showed a statistically significant relationship between air fluoride and bone density increases. In addition, urinary fluoride levels were greater in the 17 individuals with greatest exposure compared to the remaining 57 workers (average = 5.18 mg F/L vs. 4.53 mg F/L). No differences between exposed and unexposed individuals were observed for gastrointestinal, cardiovascular, or hematologic systems, or in a physical exam. A significant ($p < 0.05$) increase in the incidence of historical acute respiratory disease was observed in fluoride-exposed individuals, however radiographic examination revealed a difference of lesser significance ($p < 0.10$) for pulmonary changes.

Largent *et al.* (1951) found significant increase in bone density in the lower thoracic spine, with calcification extending into the lateral ligaments of 3 workers exposed for 17, 14, and 10 years to HF (concentrations not estimated).

A group of 74 men who were occupationally exposed to unspecified concentrations of HF for an average of 2.7 years reported occasions of upper respiratory irritation (Evans, 1940). Repeated chest X-rays over a 5-year period did not reveal any visible evidence of lung changes. The death rate of these workers from pneumonia and other pulmonary infections was the same as that of unexposed plant employees.

The possible effects of HF on a population of 47 workers exposed to HF (concentrations unspecified) included back pain and stiffness, cervical spine, knee pain, and shortness of breath on exertion (Peperkorn and Kahling, 1944). Many workers had external HF burn scars and rigidity in the chest. Radiologic examination revealed skeletal fluorosis in 34 of the 47 workers. The first evidence of these osseous changes were in the pelvis and lumbar spine, followed by changes in the spinal column and ribs. Extremities were affected last. The degree of radiologic changes increased with duration of employment. First-degree radiologic changes (increased bone density and thickened and misshapen structure of the trabeculae with the marginal contours of the bones exhibiting slight blurring) were observed no sooner than after 3 years of employment. More severe changes took at least 7 years of employment to manifest.

Transitory hyperemia was experienced by workers in a warehouse containing HF retorts (Dale and McCauley, 1948). Twenty four of the 40 workers had definite changes in the thickness and number of trabeculae in the upper and lower jaw.

Examinations of 107 potroom workers in two aluminum plants with airborne fluorides revealed 22 subjects with limited motion of the dorsolumbar spine, compared with none in a control group of 108 workers with no history of exposure to fluorides (Kaltreider *et al.*, 1972). In one plant, 76 of 79 workers had increased bone density as measured by roentgenogram, with diagnosis of slight to moderate fluorosis. Moderate and marked fluorosis was observed after 15 years employment. The 8-hour time-weighted average fluoride content in these workplaces was 2.4 to 6.0 mg/m^3. Balazova (1971) measured significant fluoride uptake and distribution in children living near an aluminum smelter but reported no incidence of fluorosis.

V. Effects of Chronic Exposures to Animals

Stokinger (1949) studied the subchronic effects of HF inhalation in several animal species. Animals (dogs, rabbits, rats, guinea pigs, and mice; 1 to 6 per group) were exposed to 0, 7.2 mg/m^3, or 25.1 mg/m^3 6 hours/day, 6 days/week, for 30 days. Mortality, body weight, blood coagulation mechanisms, and gross pathology were measured. Exposure to 25.1 mg/m^3 for 30 days resulted in degenerative testicular changes and ulceration of the scrotum in all 4 dogs and hemorrhage and edema in the lungs of 3 dogs. Pulmonary hemorrhage was also seen in 20 of 30 rats, and 4 of 10 rabbits. Renal cortical degeneration was observed in 27 of 30 rats. All of the rats and mice at the 25.1 mg/m^3 concentration died. No mortality was observed in the other species tested. Blood fibrinogen levels were significantly increased in dogs, rats and rabbits exposed to 25.1 mg/m^3. Exposure to 7.2 mg/m^3 resulted in pulmonary hemorrhage in 1 out of 5 dogs. No other significant effects were observed at the lower concentration.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Derryberry *et al.* (1963)
Study population	74 fertilizer plant workers (67 unexposed control subjects)
Exposure method	Occupational
Critical effects	Increased bone density (skeletal fluorosis)
LOAEL	1.89 mg F/m^3 (2.46 mg HF/m^3)
NOAEL	1.07 mg F/m^3 (1.13 mg HF/m^3)
Exposure continuity	8 hours/day, 5 days/week
Exposure duration	14.1 years (range = 4.5 to 25.9 years)
Average exposure concentration	0.27 mg HF/m^3 or 0.26 mg F/m^3
Human equivalent concentration	0.27 mg HF/m^3 or 0.26 mg F/m^3
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	10
Inhalation reference exposure level for F or HF	0.03 mg HF/m^3 (30 µg HF/m^3; 0.04 ppm; 40 ppb) 0.03 mg F/m^3 (30 µg F/m^3; 0.04 ppm; 40 ppb)

No studies regarding the chronic irritant or respiratory effects of HF exposure in humans or animals were available.

Changes in bone density in association with fluoride exposure have been observed in several studies, and appear to be the most sensitive health effect for chronic exposure. The increased bone density in the Derryberry study was significantly ($p < 0.04$, Fisher's Exact Test) associated with "other osseous changes" which reportedly included disc lesions, arthritis, and calcified ligaments. An increase in pulmonary changes in the workers with high bone density was

marginally significant ($p < 0.06$) and included emphysema, fibrosis, and healed tuberculous lesions. Although dental fluorosis is a sensitive endpoint in many fluoride studies, the dental examinations of exposed workers in this study showed healthier teeth than in controls. The increased bone density observed was considered as indicating adverse effects had occurred, based on the adverse effects associated with the increased density in the study, and on research showing increased bone density caused by fluoride exposure also leads to decreased bone strength and increased fragility (Riggs *et al.*, 1990). Symptoms of abdominal pain, backache, restricted joint movement and respiratory symptoms have been associated with airborne fluoride exposures and bone density increases in industrial settings (Zhiliang et al., 1987).

It has been reported that absorption of particulate and gaseous fluorides is similar (Collings *et al.*, 1951). Therefore, it would be expected that the effects on bone density would be similar regardless of the form of fluoride. The raw data from the Derryberry *et al.* (1963) study are shown in Table 1. A Pearson correlation matrix of the variables measured in the Derryberry *et al.* study indicated that bone density was best correlated with mean air fluoride level, and to a lesser extent with the age of the individual. A log-logistic regression using the log air fluoride concentration as the independent variable showed a significant ($p < 0.033$) relationship between increasing air fluoride concentrations and probability of skeletal fluorosis. The parameters for the regression were $\beta_0 = -2.3468$ (std. error = 0.6462), and $\beta_1 = 1.1736$ (std error = 0.5508); the odds ratio for the occurrence of skeletal fluorosis was 3.24. Years of exposure was not correlated with increased bone-density, according to a Pearson Correlation procedure ($p = 0.63$). Bone density has been shown to decrease with age after the age of 40 among normal, non-fluoride-exposed males (Runge *et al.*, 1979). As expected, age was very highly correlated with years exposed ($p<0.00001$), therefore including years exposed in the dose-metric likely introduces a confounding variable. Similarly, Runge *et al.* (1979) found no association between years exposed and mineral content or bone width among 245 aluminum smelter workers exposed to 2.75 or 3.2 mg F/m^3. For these reasons, years exposed was not used as the dose-metric for bone-density in this analysis.

Although a threshold was not readily apparent from the logistic regression model, grouping the 74 individuals by air fluoride exposure level into quintiles of 15 each with one group of 14, allowed for a comparison of group mean responses (Table 2). The 14 employees exposed to a time-weighted average concentration of 1.07 mg F/m^3 did not exhibit bone density changes. An analysis of the grouped responses using a binomial distribution showed a probability of $p = 0.008$ for obtaining 4/15 increased bone density observations in the 2.34 mg/m^3 group, and a probability of $p = 0.047$ for obtaining 3/15 positive observations in the 1.89 mg F/m^3 group. The 1.89 mg F/m^3 group was therefore considered a LOAEL for chronic skeletal fluorosis, and the 1.07 mg/m^3 group was considered a NOAEL. The above probabilities assume that a chance occurrence is, at most 1 in 18 of skeletal fluorosis or other cause leading to an abnormally dense x-ray in the general population. Since osteosclerosis is a rare condition that is associated with several types of hematological malignancies such as myeloid leukemia, the actual incidence of conditions leading to osteosclerosis is far below 1 in 18. This lends strong support to the consideration of 1.89 mg/m^3 as a LOAEL for skeletal fluorosis.

The major strengths of the key study are the observation of health effects in a large group of workers exposed over many years, the availability of individual exposure estimates for each worker, and the identification of a NOAEL. The primary uncertainty in the study is the lack of a comprehensive health effects examination. Another source for potential concern is the relative susceptability of children to the effects of inhaled fluorides, considering the rapid bone growth in early years. Although a number of studies were located that compared children and adult responses to environmental sources of fluorides, none of the differences in fluorosis were of a sufficient magnitude to warrant a greater than 10-fold uncertainty factor for individual susceptability.

Table 1. Data on worker exposure to fluoride from Derryberry *et al.* (1963)

Obsv. #	ID	Bone density	Years exposed	Urine max F (mg F/L)	Urine min F (mg F/L)	Mean urinary F (mg F/L)	Age (years)	Air fluoride (mg/m^3)	*OEHHA exposure grouping*
1	119	normal	18.5	43.0	2.8	14.7	58	8.16	5
2	0	normal	8.4	24.7	5.3	9.6	42	3.19	4
3	41	normal	15.8	35.0	2.5	9.1	35	3.29	4
4	147	high	9.6	17.1	2.1	8.9	60	5.98	5
5	120	normal	16.7	20.5	3.4	8.6	55	3.29	4
6	54	high	17.0	44.0	4.0	8.6	56	7.73	5
7	148	normal	10.5	14.0	3.7	8.4	41	8.32	5
8	314	high	14.4	22.7	1.7	8.3	56	3.24	4
9	29	normal	17.0	18.2	2.5	7.7	50	2.60	3
10	14	normal	14.3	19.4	2.1	6.3	46	2.33	3
11	115	normal	15.2	18.5	1.4	6.3	38	2.11	3
12	10	high	10.3	22.0	2.3	6.1	38	2.72	4
13	4	high	7.1	7.7	2.0	5.7	54	3.22	4
14	51	normal	14.9	42.0	0.8	5.6	46	3.18	4
15	94	normal	16.2	15.4	3.3	5.5	56	5.12	5
16	217	normal	7.1	7.1	2.6	5.3	42	2.54	3
17	281	high	7.8	8.6	1.1	5.2	36	3.79	4
18	114	normal	10.4	13.2	2.8	5.2	38	7.66	5
19	7	normal	7.8	9.1	2.2	5.1	43	2.91	4
20	308	normal	11.9	6.7	3.5	5.1	44	1.89	2
21	301	high	15.2	9.5	2.5	5	36	2.56	3
22	72	normal	25.9	13.7	2.1	4.9	55	5.55	5
23	241	high	17.0	10.0	1.9	4.9	46	4.48	5
24	345	normal	10.5	7.1	2.0	4.9	47	1.49	1
25	26	normal	16.4	12.2	0.5	4.7	39	2.41	3
26	231	high	16.3	8.2	2.8	4.6	62	1.88	2
27	2	normal	24.7	8.9	2.1	4.6	46	3.53	4
28	295	normal	14.5	10.7	0.9	4.6	44	2.07	3
29	1	normal	8.9	5.9	2.4	4.5	30	1.92	2
30	203	high	18.2	6.8	1.6	4.4	43	2.66	3
31	63	normal	16.2	7.4	2.0	4.3	55	3.90	5
32	5	normal	4.5	11.5	1.9	4.3	43	1.12	1
33	460	normal	12.5	6.1	1.6	4.3	60	2.13	3
34	249	high	15.0	8.0	1.8	4.3	39	2.95	4
35	3	normal	7.6	14.5	2.1	4.3	31	3.90	5
36	322	normal	9.3	6.3	2.0	4.3	35	4.23	5
37	8	high	24.8	5.9	3.0	4.2	55	2.50	3

Observation	ID	Bone density	Years exposed	Urine max F (mg F/L)	Urine min F (mg F/L)	Mean urinary F (mg F/L)	Age (years)	Air fluoride (mg F/m^3)	*OEHHA exposure grouping*
38	3	normal	15.2	12.2	2.1	4.2	42	1.14	1
39	309	normal	12.1	5.5	2.4	4.1	42	1.94	2
40	36	normal	9.1	13.2	0.8	4.1	33	1.94	2
41	45	normal	11.3	14.0	2.2	4.1	33	3.84	4
42	70	normal	17.9	8.0	1.0	3.9	44	4.00	5
43	250	high	9.8	6.7	1.5	3.9	35	1.78	2
44	38	normal	16.9	5.9	1.0	3.9	35	2.10	3
45	200	high	14.0	7.0	2.8	3.8	66	3.92	5
46	183	normal	9.8	4.9	2.2	3.7	48	1.67	2
47	32	normal	12.5	6.6	0.9	3.7	47	2.21	3
48	25	normal	13.6	5.5	1.5	3.7	44	1.86	2
49	21	normal	13.9	9.1	0.4	3.7	50	1.98	2
50	304	normal	13.4	5.0	2.1	3.7	36	2.62	3
51	132	normal	10.9	5.1	2.4	3.6	39	1.81	2
52	6	high	8.4	4.8	0.9	3.6	35	3.85	5
53	244	normal	16.6	7.1	1.4	3.6	62	2.87	4
54	30	normal	14.0	14.0	0.9	3.6	43	1.56	1
55	88	high	15.5	4.9	1.7	3.5	66	2.06	2
56	227	normal	16.6	5.7	1.0	3.5	41	1.18	1
57	271	normal	17.7	4.1	3.0	3.4	60	1.82	2
58	19	normal	13.9	10.0	1.8	3.4	41	1.32	1
59	190	normal	9.3	7.7	1.9	3.3	36	1.95	2
60	258	normal	17.8	5.6	1.6	3.2	58	0.87	1
61	278	normal	10.0	7.0	0.3	3.2	34	1.93	2
62	331	normal	12.8	5.6	1.5	3.1	34	1.23	1
63	91	normal	25.3	7.9	0.2	3.1	63	3.49	4
64	342	normal	18.5	6.0	1.3	3	40	2.73	4
65	261	normal	18.1	5.3	0.9	2.9	52	4.41	5
66	291	normal	13.5	4.5	1.5	2.8	34	2.14	3
67	149	normal	11.3	4.5	2.1	2.8	34	0.76	1
68	2	normal	24.7	4.5	1.5	2.7	51	1.15	1
69	4	normal	16.8	5.7	1.2	2.7	56	0.71	1
70	109	normal	8.3	5.1	0.8	2.7	36	1.89	2
71	242	normal	18.1	4.1	1.2	2.5	49	1.26	1
72	179	normal	18.9	3.9	1.0	2.4	46	0.50	1
73	325	high	11.8	5.0	0.5	2.2	40	2.10	3
74	159	normal	18.9	5.0	0.7	2.1	45	0.67	1

Table 2. Grouped mean exposure

Exposure group	Mean age ± SD	Mean air level mg F/m^3 ± SD	Number of responses	Probability of difference from group 1*
1	45.0 ± 7.0	1.07 ± 0.32	0/14**	Not Applicable
2	43.9 ± 11.2	1.89 ± 0.09	3/15***	0.047
3	43.0 ± 7.6	2.34 ± 0.23	4/15	0.008
4	45.9 ± 9.8	3.22 ± 0.35	5/15	0.001
5	48.5 ± 10.7	5.41 ± 1.72	5/15	0.001

* Probability of obtaining result assuming a chance occurrence of abnormally dense x-ray of, at most, 1 in 18 individuals, using a binomial distribution (Systat for Windows v.5.05, 1994).
** NOAEL
*** LOAEL ($p < 0.05$)

VI. References

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CHRONIC TOXICITY SUMMARY

FORMALDEHYDE

(Methanal; Oxoymethane; Oxomethylene; Methylene oxide; Formic aldehyde; Methyl aldehyde)

CAS Registry Number: 50-00-0

I. Chronic Toxicity Summary

Inhalation reference exposure level	**2 μg/m^3**
Critical effect(s)	Upper and lower airway irritation; eye irritation in humans (occupational)
Hazard index target(s)	Respiratory system; eyes

II. Physical and Chemical Properties (HSDB, 1994)

Molecular formula	CH_2O
Molecular weight	30.03 g/mol
Description	Colorless gas
Specific gravity	0.815 @ -20°C
Boiling point	-19.5°C
Vapor pressure	1.08 torr @ 26.1°C
Solubility	Soluble in water, ethanol, ether, other polar solvents
Conversion factor	1 ppm = 1.5 mg/m^3 @ 25° C

III. Major Uses or Sources (CARB, 1992; HSDB, 1995)

Formaldehyde is used in the manufacture of melamine, polyacetal, and phenolic resins. Phenol-formaldehyde resins are used in the production of plywood, particleboard, foam insulation, and a wide variety of molded or extruded plastic items. Formaldehyde is also used as a preservative, a hardening and reducing agent, a corrosion inhibitor, a sterilizing agent, and in embalming fluids. Indoor sources include upholstery, permanent press fabrics, carpets, pesticide formulations, and cardboard and paper products. Outdoor sources include emissions from fuel combustion (motor vehicles), industrial fuel combustion (power generators), oil refining processes, and other uses (copper plating, incinerators, etc.).

IV. Effects of Human Exposure

Formaldehyde primarily affects the mucous membranes of the upper airways and eyes. Exposed populations that have been studied include embalmers, residents in houses insulated with urea-formaldehyde foam, anatomy class students, histology technicians, wood and pulpmill workers, and asthmatics. The voluminous body of data describing these effects has been briefly summarized below. For the sake of brevity, only the studies that best represent the given effects are presented.

Kerfoot and Mooney (1975) reported that estimated formaldehyde exposures of 0.25-1.39 ppm evoked numerous complaints of upper respiratory tract and eye irritation among 7 embalmers at 6 different funeral homes. Three of the 7 embalmers in this study reportedly had asthma. Levine *et al.* (1984) examined the death certificates of 1477 Ontario undertakers. Exposure measurements taken from a group of West Virginia embalmers were used as exposure estimates for the embalming process, ranging from 0.3-0.9 ppm (average 1-hour exposure) and 0.4-2.1 ppm (peak 30-minute exposure). Mortality due to non-malignant diseases was significantly elevated due to a two-fold excess of deaths related to the digestive system. The authors suggest increased alcoholism could have contributed to this increase.

Ritchie and Lehnen (1987) reported a dose-dependent increase in health complaints (eye and throat irritation, and headaches) in 2000 residents living in 397 mobile and 494 conventional homes, that was demonstrated by logistic regression. Complaints of symptoms of irritation were noted at concentrations of 0.1 ppm formaldehyde or above. Similarly, Liu *et al.* (1991) found that exposure to 0.09 ppm (0.135 mg/m^3) formaldehyde exacerbated chronic respiratory and allergy problems in residents living in mobile homes.

Employees of mobile day-care centers (66 subjects) reported increased incidence of eye, nose and throat irritation, unnatural thirst, headaches, abnormal tiredness, menstrual disorders, and use of analgesics as compared to control workers (Olsen *et al.*, 1982). The mean formaldehyde concentration in these mobile units was 0.29 ppm (0.43 mg/m^3) (range = 0.24 - 0.55 mg/m^3). The exposed workers were exposed in these units a minimum of 3 months. A control group of 26 subjects in different institutions was exposed to a mean concentration of 0.05 ppm (0.08 mg/m^3) formaldehyde.

Occupants of houses insulated with urea-formaldehyde foam insulation (UFFI) (1726 subjects) were compared with control subjects (720 subjects) for subjective measures of irritation, pulmonary function (FVC, FEV_1, FEF_{25-75}, FEF_{50}), nasal airway resistance, odor threshold for pyridine, nasal cytology, and hypersensitivity skin-patch testing (Broder *et al.*, 1988). The mean length of time of exposure to UFFI was 4.6 years. The mean concentration of formaldehyde in the UFFI-exposed group was 0.043 ppm, compared with 0.035 ppm for the controls. A significant increase in symptoms of eye, nose and throat irritation was observed in subjects from UFFI homes, compared with controls. No other differences from control measurements were observed.

An increase in severity of nasal epithelial histological lesions, including squamous epithelium, keratosis, and metaplasia of the nasal epithelium was observed in 75 wood products workers exposed to between 0.1 and 1.1 mg/m^3 formaldehyde for a mean duration of 10.5 years (range = 1 - 39 years) compared to an equal number of control subjects (Edling *et al.*, 1988).

Alexandersson and Hedenstierna (1989) evaluated symptoms of irritation, spirometry, and immunoglobulin levels in 34 wood workers exposed to formaldehyde over a 4-year period. Exposure to 0.4 - 0.5 ppm formaldehyde resulted in significant decreases in FVC, FEV_1, and FEF_{25-75}. Removal from exposure for 4 weeks allowed for normalization of lung function in the non-smokers.

Kriebel *et al.* (1993) conducted a subchronic epidemiological study of 24 anatomy class students exposed to a range of formaldehyde of 0.49 to 0.93 ppm (geometric mean = 0.73 $\pm$ 1.22 ppm) for 3 hours per week for 10 weeks. One subject was a smoker, 2 reported current asthma, and 3 reported childhood asthma without current symptoms. Eye and throat irritation was observed to be significantly elevated in the students after classes compared with pre-laboratory session exposures. In addition, peak expiratory flow measurements declined by an average of 10 L/minute to 2% of baseline, but returned to normal after 14 weeks of non-exposure.

Histology technicians (280 subjects) were shown to have reduced pulmonary function , as measured by FVC, FEV_1, FEF_{25-75}, and FEF_{75-85}, compared with 486 controls (Kilburn *et al.*, 1989). The range of formaldehyde concentrations was 0.2 - 1.9 ppm, volatilized from formalin preservative solution.

Malaka and Kodama (1990) investigated the effects of formaldehyde exposure in plywood workers (93 exposed, 93 controls) exposed for 26.6 years, on average, to 1.13 ppm (range = 0.28 - 3.48 ppm). Fifty-three smokers were present in both study groups. Exposure assessment was divided into 3 categories: high (> 5 ppm), low (< 5 ppm), and none (reference group). Subjective irritation and pulmonary function tests were performed on each subject, and chest x-rays were conducted on 10 randomly selected volunteers from each group. Respiratory symptoms of irritation were found to be significantly increased in exposed individuals, compared with controls. In addition, exposed individuals exhibited significantly reduced FEV_1, FEV_1/FVC, and $FEF_{25-75,}$ compared with controls. Forced vital capacity was not significantly reduced. Pulmonary function was not found to be different after a work shift, compared to the same measurement taken before the shift. No differences in chest x-rays were observed between exposed and control workers.

Occupational exposure to formaldehyde concentrations estimated to be 0.025 ppm (0.038 mg/m^3) for greater than 6 years resulted in complaints by 22 exposed workers of respiratory, gastrointestinal, musculoskeletal, and cardiovascular problems, and in elevated formic acid excretion in the urine (Srivastava *et al.*, 1992). A control group of 27 workers unexposed to formaldehyde was used for comparison. A significantly higher incidence of abnormal chest x-rays was also observed in formaldehyde-exposed workers compared with controls.

Chemical plant workers (70 subjects) were exposed to a mean of 0.17 ppm (0.26 mg/m^3) formaldehyde for an unspecified duration (Holmstrom and Wilhelmsson, 1988). Compared with 36 control workers not exposed to formaldehyde, the exposed subjects exhibited a higher frequency of eye, nose, and deep airway discomfort. In addition, the exposed subjects had diminished olfactory ability, delayed mucociliary clearance, and decreased FVC.

Alexandersson *et al.* (1982) compared the irritant symptoms and pulmonary function of 47 carpentry workers exposed to a mean concentration of formaldehyde of 0.36 ppm (range = 0.04 - 1.25 ppm) with 20 unexposed controls. The average length of employment for the exposed workers was 5.9 years. Symptoms of eye and throat irritation as well as chest oppression were more common in exposed workers. In addition, a significant reduction in FEV_1, FEV_1/FVC, and MMF was observed in exposed workers, as compared with controls.

Horvath *et al.* (1988) compared subjective irritation and pulmonary function in 109 workers exposed to formaldehyde with similar measures in a control group of 254 subjects. The formaldehyde concentrations for the exposed and control groups were 0.69 ppm (1.04 mg/m^3) and 0.05 ppm (0.08 mg/m^3), respectively. Ambient outdoor concentrations of formaldehyde were 0.04 ppm (0.06 mg/m^3). Duration of formaldehyde exposure was not stated. Subjects were evaluated pre- and post workshift and compared with control subjects. Significant differences in symptoms of irritation, FEV_1, FEV_1/FVC ratio, FEF_{50}, FEF_{25}, and FEF_{75} were found when comparing exposed subjects' pre- and post workshift values. However, the pre-workshift values were not different from controls.

The binding of formaldehyde to endogenous proteins creates haptens which can elicit an immune response. Chronic exposure to formaldehyde has been associated with immunological hypersensitivity as measured by elevated circulating IgG and IgE autoantibodies to human serum albumin (Thrasher *et al.*, 1987). In addition, a decrease in the proportion of T-cells was observed, indicating altered immunity. Thrasher *et al.* (1990) later found that long-term exposure to formaldehyde was associated with autoantibodies, immune activation, and formaldehyde-albumin adducts in patients occupationally exposed, or residents of mobile homes or of homes containing particleboard sub-flooring. The authors suggest that the hypersensitivity induced by formaldehyde may account for a mechanism for asthma and other health complaints associated with formaldehyde exposure.

Symptoms of irritation were reported by 66 workers exposed for 1 - 36 years (mean = 10 years) to a mean concentration of 0.17 ppm (0.26 mg/m^3) formaldehyde (Wilhelmsson and Holmstrom, 1992). Controls (36 subjects) were exposed to a mean concentration of 0.06 ppm (0.09 mg/m^3) formaldehyde. The significant increase in symptoms of irritation in exposed workers did not correlate with total serum IgE antibody levels. However, 2 exposed workers, who complained of nasal discomfort, had elevated IgE levels. In another occupational health study, 37 workers who were exposed for an unspecified duration to formaldehyde concentrations in the range of 0.003 to 0.073 ppm, reported ocular irritation, however no significant serum levels of IgE or IgG antibodies to formaldehyde-human serum albumin were detected (Grammer *et al.*, 1990).
An epidemiological study of the effects of formaldehyde on 367 textile and shoe manufacturing workers employed for a mean duration of 12 years showed no significant association between

formaldehyde exposure, pulmonary function (FVC, FEV_1, and PEF) in normal or asthmatic workers, and occurrence of specific IgE antibodies to formaldehyde (Gorski and Krakowiak, 1991). The concentrations of formaldehyde tested did not exceed 0.5 ppm (0.75 mg/m^3).

Workers (38 total) exposed for a mean duration of 7.8 years to 0.11 - 2.12 ppm (mean = 0.33 ppm) formaldehyde were studied for their symptomatology, lung function, and total IgG and IgE levels in the serum (Alexandersson and Hedenstierna, 1988). The control group consisted of 18 unexposed individuals. Significant decrements in pulmonary function (FVC and FEV_1) were observed, compared with the controls. Eye, nose, and throat irritation was also reported more frequently with the exposed group, compared with the control group. No correlation was found between duration of exposure, or formaldehyde concentration and presence of IgE and IgG antibodies.

The effects of formaldehyde on asthmatics appears to be dependent on previous, repeated exposure to formaldehyde. Burge *et al.* (1985) found that 3 out of 15 occupationally exposed workers challenged with formaldehyde vapors at concentrations from 1.5 ppm to 20.6 ppm for brief durations exhibited late asthmatic reactions. Six other subjects had immediate asthmatic reactions likely due to irritant effects. Asthmatic responses (decreased PEF, FVC, and FEV_1) were observed in 12 occupationally-exposed workers challenged with 1.67 ppm (2.5 mg/m^3) formaldehyde (Nordman *et al.*, 1985). Similarly, asthmatic responses were observed in 5 of 28 hemodialysis workers occupationally exposed to formalin and challenged with formaldehyde vapors (concentration not measured) (Hendrick and Lane, 1977). In asthmatics not occupationally exposed to formaldehyde, Sheppard *et al.* (1984) found that a 10-minute challenge with 3 ppm formaldehyde coupled with moderate exercise did not induce significant changes in airway resistance or thoracic gas volume.

V. Effects of Animal Exposure

Fischer-344 rats and B6C3F1 mice (120 animals/sex) were exposed to concentrations of 0, 2.0, 5.6, or 14.3 ppm formaldehyde vapor for 6 hours/day, 5 days/week for 24 months (Kerns *et al.*, 1983). The exposure period was followed by up to 6 months of non-exposure. Interim sacrifices were conducted at 6, 12, 18, 24, 27, and 30 months. Both male and female rats in the 5.6 and 14.3 ppm groups demonstrated decreased body weights over the 2-year period. At the 6 month sacrifice, the rats exposed to 14.3 ppm formaldehyde had non-neoplastic lesions of epithelial dysplasia in the nasal septum and turbinates. As the study progressed, epithelial dysplasia, squamous dysplasia, and mucupurulent rhinitis increased in severity and distribution in all exposure groups. In mice, cumulative survival decreased in males from 6 months to the end of the study. Serous rhinitis was detected at 6 months in the 14.3 ppm group of mice. Metaplastic and dysplastic changes were noted at 18 months in most rats in the 14.3 ppm group and in a few mice in the 5.6 ppm exposure group. By 24-months, the majority of mice in the 14.3 ppm group had metaplastic and dysplastic changes associated with serous rhinitis, in contrast to a few mice in the 5.6 ppm group and a few in the 2 ppm group (exact number not given).

Rusch *et al.* (1983) exposed groups of 6 male cynomolgus monkeys, 20 male or female rats, and 10 male or female hamsters to 0, 0.2, 1.0, or 3.0 ppm (0, 0.24, 1.2, or 3.7 mg/m^3) formaldehyde vapor for 22 hours/day, 7 days/week for 26 weeks. There was no treatment-related mortality during the study. In monkeys, the most significant findings were hoarseness, congestion and squamous metaplasia of the nasal turbinates in 6/6 monkeys exposed to 2.95 ppm. There were no signs of toxicity in the lower exposure groups. In the rat, squamous metaplasia and basal cell hyperplasia of the nasal epithelia were significantly increased in rats exposed to 2.95 ppm. The same group exhibited decreased body weights and decreased liver weights. In contrast to monkeys and rats, hamsters did not show any signs of response to exposure, even at 2.95 ppm.

Wilmer *et al.* (1989) found that intermittent (8 hours/day, 5 days/week) exposures of rats to 4 ppm formaldehyde for 13 weeks resulted in significant histological changes in the nasal septum and turbinates. In contrast, continuous exposure of rats for 13 weeks to 2 ppm formaldehyde did not produce significant lesions. Similarly, Appelman *et al.* (1988) found significant nasal lesion in rats (20 per group; 0, 0.1, 1.0, or 10.0 ppm) exposed to 10 ppm formaldehyde 6 hours/day, 5 days/week for 52 weeks, but exposure to 1.0 ppm or less for this period did not result in nasal histological lesions. However, the rats exposed to formaldehyde displayed decreased body weight in all groups compared with controls.

Apfelbach and Weiler (1991) determined that rats (5 exposed, 10 controls) exposed to 0.25 ppm (0.38 mg/m^3) formaldehyde for 130 days lost the olfactory ability to detect ethyl acetate odor.

Wouterson *et al.* (1987) exposed rats (20 per group) to 0, 1, 10, or 20 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks. Rats exposed to 20 ppm displayed retarded growth, yellowing of the fur, and significant histological lesions in the respiratory epithelium. Exposure to 10 ppm did not affect growth, but resulted in significant histological lesions in the respiratory tract. No effects on specific organ weights, blood chemistries, liver glutathione levels, or urinalysis were detected at any level. No significant adverse effects were seen at the 1.0 ppm exposure level.

Maronpot *et al.* (1986) exposed groups of 20 mice to 0, 2, 4, 10, 20, or 40 ppm formaldehyde 6 hours/day, 5 days/week, for 13 weeks. Histological lesions in the upper respiratory epithelium were seen in animals exposed to 10 ppm or greater. Exposure to 40 ppm was lethal to the mice.

A six-month exposure of rats to 0, 0.5, 3, and 15 ppm formaldehyde (3 rats per group) resulted in significantly elevated total lung cytochrome P450 in all formaldehyde-exposed groups (Dallas *et al.*, 1989). The degree of P450 induction was highest after 4 days exposure and decreased slightly over the course of the experiment.

A developmental toxicity study on formaldehyde was conducted by Martin (1990). Pregnant rats (25 per group) were exposed to 0, 2, 5, or 10 ppm formaldehyde for 6 hours/day, during days 6-15 of gestation. Although exposure to 10 ppm formaldehyde resulted in reduced food consumption and body weight gain in the maternal rats, no effects on the number, viability or normal development of the fetuses was seen. In addition, Saillenfait *et al.* (1989) exposed pregnant rats (25 per group) to 0, 5, 10, 20, or 40 ppm formaldehyde from days 6 - 20 of

gestation. Maternal weight gain and fetal weight were significantly reduced in the 40 ppm exposure group. No significant fetotoxicity or teratogenic defects were observed.

VI. Derivation of Chronic Reference Exposure Level (REL)

Studies	Wilhelmsson and Holmstrom, 1992; Holmstrom and Wilhelmsson, 1988
Study population	Human chemical plant workers (66 subjects)
Exposure method	Discontinuous occupational exposure
Critical effects	Nasal and eye irritation, nasal obstruction, and lower airway discomfort
LOAEL	Mean of 0.26 mg/m^3 (range = 0.05 to 0.6 mg/m^3) (described as exposed group)
NOAEL	Mean of 0.09 mg/m^3 (described as control group)
Exposure continuity	8 hours/day, 5 days/week (assumed)
Exposure duration	10 years (average); range = 1-36 years
Average occupational concentration	0.021 mg/m^3 for LOAEL group
Human equivalent concentration	0.021 mg/m^3
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	10
Inhalation reference level	0.002 mg/m^3 (2 $\mu g/m^3$; 0.001 ppm; 1 ppb)

Sixty six workers in a formaldehyde-producing plant were surveyed for symptoms of upper respiratory and eye irritation, in addition to examination of serum IgE antibodies to formaldehyde. Sixty six percent of the non-atopic workers experienced general nasal discomfort compared with only 17% discomfort in the 36 controls ($p < 0.001$). In addition, the workers exposed to formaldehyde experienced significantly increased incidence of lower airway discomfort as measured by cough, wheezing, and symptoms of bronchitis ($p < 0.01$). Formaldehyde-exposed workers also had significantly increased incidence of annoying dermatitis (39%) compared with controls (8%, $p < 0.01$). Twenty four percent of the formaldehyde-exposed workers also complained of eye irritation, compared with 6% in the control group ($p < 0.05$).

The study by Horvath et al (1988) supports the results of the study by Wilhelmsson and Holmstrom (1992) and can be used to further support the selection of the 0.09 mg/m^3 value as a NOAEL for irritation. The 254 control subjects in the Horvath *et al.* (1988) study were exposed to a mean concentration of 0.05 ppm (0.08 mg/m^3). The ambient outdoor concentration of formaldehyde in this study was 0.04 ppm (0.06 mg/m^3). The prevalence of symptoms in the 2 control groups appears to be similar (e.g. 17% general nasal discomfort vs. 14.2% stuffy nose, 2% burning of the nose, and 8% itching of the nose). In the Horvath *et al.* study, concentrations ranging from ambient to 0.05 ppm (0.08 mg/m^3) were considered the baseline for exposure comparisons.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years and the observation of a NOAEL. The major areas of uncertainty is the uncertainty in estimating exposure and the potential variability in exposure concentration.

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CHRONIC TOXICITY SUMMARY

GLUTARALDEHYDE

(1,5-pentanedial; 1,5-pentanedione; glutaric dialdehyde; Aldesen; Cidex; Sonacide)

CAS Registry Number: 111-30-8

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.1 µg/m^3**
Critical effect(s)	Neutrophilic infiltration in the olfactory epithelium of the respiratory system of mice
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1995*)*

Molecular formula	$C_5H_8O_2$
Molecular weight	100.13 g/mol
Description	Colorless liquid
Vapor pressure	17mm Hg 20°C
Solubility	Soluble in water, alcohol, benzene
Conversion factor	4.1 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Glutaraldehyde is a chemical frequently used as a disinfectant and sterilizing agent against bacteria and viruses (2% solution), an embalming fluid and tissue fixative, a component of leather tanning solutions, and an intermediate in the production of certain sealants, resins, dyes, and electrical products (HSDB, 1995). For commercial purposes, solutions of 99%, 50%, and 20% are available.

IV. Effects of Human Exposure

Evidence of the toxicity of glutaraldehyde to humans is limited to reports of occupational exposure from its use as a disinfectant and sterilizing agent. Frequently observed effects from exposure include skin sensitivity resulting in dermatitis, and irritation of the eyes and nose with accompanying rhinitis (Jordan *et al.*, 1972; Corrado *et al.*, 1986; Hansen, 1983; Wiggins *et al.*, 1989). Occupational asthma has also been reported among workers repeatedly exposed to glutaraldehyde, particularly respiratory technologists who use glutaraldehyde as a sterilizing agent for endoscopes (Chan-Yeung *et al.*, 1993; Stenton *et al.*, 1994; Gannon *et al.*, 1995). No

studies addressing glutaraldehyde sensitivity from chronic exposure include the quantitation of the exposure levels which led to the sensitization.

V. Effects of Animal Exposure

The histopathology of the respiratory tract in rats and mice exposed to glutaraldehyde by inhalation was examined (Gross *et al.*, 1994). F344 rats and B6C3F1 mice (8/sex/group) were continuously exposed to glutaraldehyde in recirculating exposure chambers at concentrations of 0, 62.5, 125, 250, 500, or 1000 ppb glutaraldehyde for one day, 4 days, 6 weeks, or 13 weeks. At termination, respiratory tract tissue as well as duodenum and any gross lesions were collected and formalin fixed. Animals were treated with tritiated thymidine two hours before sacrifice to evaluate cell replication in certain respiratory tract tissues. Respiratory tract tissue sections were made as follows: transverse sections of the nose and trachea, frontal section of the carina, and longitudinal section of the lung. Ten male and 10 female mice in the 1000 ppb group and one female mouse in the 500 ppb group died during the course of the study. Two male and 3 female rats died during the course of the study. Histopathological examination of animals surviving to the end of the study entailed scoring the severity of the finding from “no response” to “very severe” response on a 0 to 5 scale. Unit length labeling index, the indicator of cell proliferation, was evaluated by autoradiography at two sites: the nasal vestibule and the dorsal atrioturbinate.

Lesions in animals treated with glutaraldehyde appeared primarily in the anterior third of the nose. Lesions were apparently more increased in mice compared to rats due to some level of “background” non-suppurative lesions in the rats. Mice were considered devoid of background lesions. In the 13-week study, female mice were the most sensitive, with lesions averaging a score of 2 (mild and clear, but of limited extent and/or severity). The lesions were characterized as neutrophilic infiltration primarily in the squamous epithelium of the vestibule, with thickening of the epithelium leading to loss of the characteristic surface grooves. Both cell size and number were reported to be increased. Lesions were generally found to increase in nature and severity with increased time and level of exposure. Obstruction of the nasal vestibule was thought to account for the mortality of animals in the higher dose groups. In female mice at 13 weeks, all glutaraldehyde dose groups showed the accumulation of eosinophilic proteinaceous deposits in the respiratory epithelium of the maxilloturbinate margin. Examination of unit length labeling indices as a measure of growth showed significant increases in all treated groups of female mice. No evidence of exposure related lesions was found in the respiratory tract in the trachea, carina, bronchi, or lungs.

Nine day and 14-week inhalation studies were conducted, exposing male and female F-344 rats to 0, 0.3, 1.1 and 3.1 ppm glutaraldehyde and 0, 0.2, 0.63, and 2.1 ppm glutaraldehyde, respectively, in the 9-day study and both sexes to 0, 21, 49, and 194 ppb glutaraldehyde in the 14 week study (animal numbers were not specified)(Greenspan *et al.*, 1985). Exposures were conducted for 6 hours per day, 5 days per week. In the 9-day study, observations in the high and intermediate dose level groups included reduced body weight gain, inflammation of the nasal and olfactory mucosa, and sensory irritation. In the two highest doses of the 14-week study, statistically significant differences in body weight gain were observed as well as perinasal

wetness. No histopathological indication of inflammation in olfactory or nasal mucosa was observed.

Mice were exposed to 0, 0.3, 1.0, and 2.6 ppm glutaraldehyde vapors for 6 hours/days for 4, 9, or 14 days (Zissu *et al.*, 1994). These mice were sacrificed immediately after the exposure period. Other groups exposed to 1.0 ppm for 14 days were sacrificed after recovery periods of 1, 2, and 4 weeks. After 4 days of exposure to the lowest dose, mice showed lesions in the respiratory epithelium of the septum, and the naso- and maxilloturbinates. After exposure to 1.0 ppm glutaraldehyde, lesions were still judged as severe after 2 weeks of recovery.

In a study comparing the effects of intra-nasally instilled glutaraldehyde and formaldehyde on rat nasal epithelium, inflammation, epithelial degeneration, respiratory epithelial hypertrophy, and squamous metaplasia were found in treated animals (St. Clair *et al.*, 1990). Acute inhalation exposure to formaldehyde produced identical lesions. Ten fold higher concentrations of instilled formaldehyde were required to produce the same effect as glutaraldehyde when instilled.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Gross *et al.*, 1994
Study population	Male and female F344 rats and B6C3F1 mice (8/sex/group)
Exposure method	Continuous inhalation exposure (0, 62.5, 125, 250, 500, or 1000 ppb)
Critical effects	Neutrophilic infiltration in olfactory epithelium
LOAEL	62.5 ppb (female mice)
NOAEL	Not observed
Exposure continuity	24 hr/day, 7 days/week
Exposure duration	13 weeks
Average experimental exposure	62.5 ppb
Human equivalent concentration	10.5 ppb (gas with extrathoracic respiratory effects, RGDR = 0.17, BW = 28 g, MV = 0.032 L/min, SA = 3 cm^2)
Subchronic uncertainty factor	3
LOAEL uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.035 ppb (0.1 $\mu g/m^3$)

Several studies indicate the upper respiratory tract is a target for the toxicity of glutaraldehyde from inhalation exposure. Reports of toxicity to humans show exposure can lead to occupational asthma as well as irritation of the eyes and nose with accompanying rhinitis. Likewise, animals exposed to glutaraldehyde by the inhalation route show evidence of respiratory irritation with the induction of lesions of the anterior nasal cavities upon long-term exposure (Gross *et al.*, 1994;

Greenspan *et al.*, 1985). The most thorough reporting of this effect is the study by Gross *et al.* (1994) showing neutrophilic infiltration in the olfactory epithelium in the lowest dose exposure group (female mice exposed to 62.5 ppb showed subepithelial neutrophilic infiltration). This level was taken to be the LOAEL. This effect on the nasal epithelium was demonstrated to be both concentration- and exposure duration-dependent.

The major strength of the inhalation REL is the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis. Major areas of uncertainty are the lack of human data, the lack of chronic inhalation exposure studies, the lack of reproductive and developmental toxicity studies, the lack of dermal sensitization studies, and the lack of observation of a NOAEL.

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CHRONIC TOXICITY SUMMARY

HEXACHLORO-1,3-BUTADIENE

(1,1,2,3,4,4-hexachloro-1,3-butadiene; hexachlorobutadiene; perchlorobutadiene; HCBD)

CAS Registry Number: 87-68-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**90 μg/m^3**
Oral reference exposure	**0.00001 mg/kg bw-day**
Critical effect(s)	Reduced maternal weight gain (inhalation exposures) Dose-dependent increase in renal tubular epithelial regeneration in B6C3F$_1$ mice, characterized by increased basophilia of the tubular cell cytoplasm, increased number of nuclei and occasional mitotic figures (oral exposures)
Hazard index target(s)	Kidney

II. Chemical Property Summary (HSDB, 1995, unless otherwise noted)

Molecular formula:	C_4Cl_6
Molecular Weight:	260.76
Description:	Clear, colorless liquid with a faint turpentine-like odor.
Vapor Pressure:	0.15 mm Hg at 25°C
Solubility:	Practically insoluble in water, 2-2.55 mg/L at 20°C (ATSDR, 1994). Soluble in ethanol and diethyl ether.
Conversion factor:	10.67 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

Prior to 1975, the largest use of hexachloro-1,3-butadiene (HCBD) in the U.S. was for recovery of 'snift' (chlorine-containing) gas in chlorine plants (HSDB, 1995). More recent information indicates that HCBD is no longer used for this process (ATSDR, 1994). Other industrial uses include applications as a solvent for many organic substances, a heat-transfer liquid in transformers, a hydraulic and gyroscope fluid, and as a chemical intermediate for fluorinated lubricants and rubber compounds. Outside of the U.S. it is used as a pesticide (ATSDR, 1994).

Since about 1974, all HCBD used commercially in the U.S. has been imported from Germany. HCBD is not known to occur as a natural product. The chemical can be released during refuse combustion and is found in fly ash. It is also a by-product from the manufacture of chlorinated hydrocarbons such as tetrachloroethylene (Yang, 1988). Environmental releases to soil or evaporation into air can occur during its various uses in industry. The highest exposure to HCBD will probably be in occupational settings while the primary exposure to the public will probably be from drinking water. A smaller population will be exposed to HCBD that is in fish and other edible aquatic organisms.

IV. Effects of Human Exposure

No data on HCBD poisoning are available in epidemiological and case reports. However, a study in Russia investigated a group of 205 vineyard workers who were exposed seasonally to both HCBD (0.8-30 mg/m^3) and polychlorobutane-80 (0.12-6.7 mg/m^3) in the air over fumigated areas (Krasniuk *et al.*, 1969). Multiple toxic effects were recorded including the development of hypotension, cardiac disease, chronic bronchitis, disturbance of nervous function and chronic hepatitis. HCBD has been detected in human adipose tissue with a concentration ranging from 0.8 to 8 µg/kg wet weight (Mes *et al.*, 1982). While this finding indicates that exposure to HCBD occurs in humans, it does not identify sources or routes of exposure.

Studies of workers exposed to various organic solvents have found raised serum bile acid concentrations despite normal values from standard liver function tests (Franco *et al.*, 1986). Changes in bile acid concentrations may reflect early and small disturbances of liver function. In a study of 35 workers chronically exposed to HCBD (0.005 to 0.02 ppm), a dose-dependent increase in concentrations of some serum bile acids with increased exposure to HCBD was found even though liver function tests were normal (Driscoll *et al.*, 1992). It should be noted that the workers were also potentially exposed to other solvents such as carbon tetrachloride and perchloroethylene. These findings support the suggestion that serum bile acids are a more sensitive indicator of changes in liver function than the standard liver function tests.

V. Effects of Animal Exposure

HCBD absorption studies were not present in the literature. However, based on results of dermal (Duprat and Gradiski, 1978) and inhalation studies (Saillenfait *et al.*, 1989), it is apparent that HCBD is well absorbed by these routes. In an oral gavage study, 72 hours after administration of 1 mg/kg [^{14}C]HCBD to rats, 5.3% of the dose was exhaled unchanged and 76% was metabolized in urine and feces or exhaled as $^{14}CO_2$ (Reichert *et al.*, 1985). After a 50 mg/kg dose of [^{14}C]HCBD, gastro-intestinal absorption appeared to be saturated with the result that unchanged HCBD constituted the major portion of the 69% of radioactivity eliminated. The kidney (outer medulla), liver and adipose tissue appeared to concentrate the labeled HCBD. Covalent binding to proteins in kidney and liver agreed well with the organ-specific toxicity of HCBD; binding was higher in the kidney, independent of dose. The two major urinary metabolites found, pentachlorobutadiene methylthio ether and pentachlorobutadiene carboxymethylthio ether,

suggested that glutathione conjugation is the first step in HCBD metabolism. Bile cannulation experiments showed that biliary excretion is the main route of excretion for HCBD (Nash *et al.*, 1984). On each of the first two days after oral dosing, 17-20% of the dose was eliminated in the bile while less than 5% of the dose was eliminated in feces. This finding indicates that enterohepatic circulation of biliary metabolites occurs. The major biliary metabolite of HCBD was a glutathione conjugate. Rats fitted with a biliary cannula were protected from kidney damage following oral dosing with HCBD, indicating that hepatic metabolites were responsible for the nephrotoxicity of HCBD. The metabolism of the glutathione conjugate of HCBD by the renal cytosolic enzyme β-lyase is thought to result in a toxic thiol that causes localized kidney damage. Metabolism and excretion of orally administered HCBD in mice produced similar results, suggesting that β-lyase-catalyzed formation of reactive intermediates from glutathione conjugates also accounts for murine nephrotoxicity (Dekant *et al.*, 1988). In a study with germ-free rats, the intestinal microflora were determined to be of little importance in detoxifying metabolites of HCBD and thereby in providing protection from nephrotoxicity (Wallin *et al.*, 1993). In rats, sex differences in HCBD biotransformation and nephrotoxicity have been recently studied (Birner *et al.*, 1995). The formation of a mercapturic acid sulfoxide metabolite by cytochrome P-450 enzymes in male rats may be responsible for the higher nephrotoxicity and slight liver damage observed in the males.

In an inhalation study investigating the potential teratogenicity of HCBD, 24 or 25 pregnant Sprague-Dawley (CD) rats/group were exposed to 0, 2, 5, 10 or 15 ppm HCBD 6 hr/day during days 6-20 of gestation (Saillenfait *et al.*, 1989). A significant reduction in maternal weight gain and in fetal body weight occurred at 15 ppm. However, no external, visceral or skeletal alterations were seen. HCBD concentrations high enough to cause maternal and slight fetal toxicity were neither embryotoxic nor teratogenic. The teratogenic potential of HCBD was also investigated in a study in which 10-15 pregnant Sprague-Dawley rats were injected intraperitoneally with 10 mg/kg body wt of HCBD on days 1 to 15 of gestation (Hardin *et al.*, 1981). This dose produced maternal toxicity (reduced body weight gain or altered weights of 2 or more organs) and fetal toxicity (delayed fetal development) without evidence of teratogenicity. In fetuses, the development of the heart was delayed 1-2 days, and dilated renal pelvises and ureters were observed.

In a 4-week feeding study that investigated the toxicity of HCBD (individually and in combination with other nephrotoxins), 5 Wistar rats/group/sex were fed diets containing 25, 100 or 400 ppm HCBD (Jonker *et al.*, 1993). The control group consisted of 10 rats/sex. Body weights were reduced significantly (10% or more) at the two highest dose levels in both sexes. Urinalysis revealed increased epithelial cells at the two highest dose levels and ketones in the 400 ppm groups. In males and females fed 400 ppm HCBD and in females fed 100 ppm HCBD, numerous clinical chemistry values in treated rats were significantly different from controls. In addition, blood urea levels were significantly reduced in exposed females even at 25 ppm. Absolute kidney weight was significantly reduced only in male rats exposed to 400 ppm HCBD in diet. Absolute liver weights were significantly reduced in males in the 400 ppm group and in females in the 100 and 400 ppm groups. Female rats also had lower absolute adrenal weights at the two highest dose levels. Microscopy revealed diffuse tubular cytomegaly in the inner cortex

of the kidneys of males and females fed 400 ppm HCBD and of females fed 100 ppm HCBD. Kidneys of males also exhibited focal necrosis in the 400 ppm group.

In a 13-week feeding study, $B6C3F_1$ mice (10/group/sex) were fed diets containing 0, 1, 3, 10, 30 or 100 ppm HCBD (NTP, 1991; Yang *et al.*, 1989). Based on estimated feed intake, the doses were equivalent to 0, 0.1, 0.4, 1.5, 4.9 and 16.8 mg/kg body wt-day for females and 0, 0.2, 0.5, 1.8, 4.5 and 19.2 mg/kg body wt-day for males. Treatment-related mortality was not observed. However, mean body weights of male mice that received 30 and 100 ppm and female mice that received 100 ppm were significantly reduced (10% or more) throughout most of the study. The most significant dose-related organ weight changes were reductions (up to 26%) of kidney weight in the three highest doses in males and the highest dose in females. A significant reduction in absolute heart weight (12%) occurred in 100 ppm dosed males. No compound-related clinical signs were observed. Microscopic examination revealed a dose-dependent increase in renal tubular epithelial regeneration, indicative of renal cellular damage, in females. The renal lesion in males was seen only at the two highest doses. The lesion was characterized by increased basophilia of the tubular cell cytoplasm, increased number of nuclei and occasional mitotic figures. Reproductive system analysis found the motility of sperm from dosed mice to be significantly lower, though not dose related, than that of controls.

In an earlier 13-week oral gavage study, 10 Wistar rats/sex/group were given 0, 0.4, 1.0, 2.5, 6.3 or 15.6 mg HCBD/kg body wt-day in oil solution (Harleman and Seinen, 1979). At the 2 highest dose levels, a significant reduction in body weight gain was observed. Females had a greater reduction in body weight gain compared to males in the 6.3 mg/kg body wt-day groups (30% vs 13%, respectively). Food consumption was also reduced (greater than 10%) at the highest dose in both sexes and in the 6.3 mg/kg body wt-day group in both sexes during the first 3 weeks. The ability to concentrate urine was significantly reduced in females at dose levels from 2.5 to 15.6 mg/kg body wt-day. The same effect was seen in males at the highest dose. Other urine and blood analyses were similar among the various groups. Relative kidney weight increases (10% or greater) were observed at the two highest doses in both sexes and relative liver weight increases (greater than 10%) observed at the highest dose in both sexes. Kidney lesions exhibited a dose-response effect in females from 2.5 to 15.6 mg/kg body wt-day and in males from 6.3 to 15.6 mg/kg body wt-day. In females at the highest dose, renal tubule epithelial cells were small, more basophilic, finely vacuolated and contained large hyperchromatic nuclei. Hypercellularity of the epithelial lining was present and the brush border was somewhat thinner or absent. Foci of necrotic cells were observed. In males, the kidney lesions were less pronounced than in females. Hepatotoxicity was noted in males at the two highest dose levels and consisted of increased basophilic, flocky granulation.

In a long-term toxicity study, 39-40 Sprague-Dawley rats/sex/group (plus 90 controls/sex) were fed diets supplying HCBD at dose levels of 0, 0.2, 2.0 or 20 mg/kg body wt-day for up to 24 months (Kociba *et al.*, 1977a,b). Multiple toxicologic effects were seen in the highest dose groups including decreased body weight gain in males (8-20%) and females (5-11%), increased mortality in males and increased secretion of coproporphyrin in both sexes. The absolute and relative kidney weight in males and relative kidney weight in females were reported as significantly greater, but data were not provided. Female rats also had lower absolute weights of

the heart and liver. Pathologic examination revealed multifocal and disseminated renal tubular epithelial hyperplasia. In rats ingesting 2.0 mg HCBD/kg body wt-day, a lower incidence of renal tubular epithelial hyperplasia was observed than at the high dose level. Urinary coproporphyrin excretion was higher in females rats at 14 months. All other toxicologic parameters at this intermediate dose level were similar to controls. Rats ingesting 0.2 mg HCBD/kg body wt-day had no treatment-related effects.

In a limited 1-generation subchronic toxicity study, groups of male and female adult rats were fed diets containing HCBD at dose levels of 0, 0.2, 2.0 or 20 mg/kg body wt-day for 90 days prior to mating, 15 days during mating, and subsequently throughout gestation and lactation for a total exposure of 19 weeks (Schwetz *et al.*, 1977; Kociba *et al.*, 1977b). There were 10 males and 12 females at the two lower dose levels plus 12 males and 24 females at the high dose level. Seventeen males and 34 females served as vehicle controls. A significant decrease in weight gain was observed in adults at the highest dose level for most of the study, but was only below 10% that of controls in females. Food consumption was reported as significantly lower in the high dose group of both sexes but amounts were not provided. However, no effect on pregnancy or neonatal survival and development was observed. In the high dose group, the body weight of neonates at the time of weaning (21 days of age) was slightly but significantly less (13%) than that of controls. Clinical chemistry determinations of adults showed no treatment-related effects. A significant decrease in heart weight (14%) in the highest dosed group of female rats was the only absolute organ weight effect following HCBD exposure. Pathologic examination revealed the kidneys from male rats at the two highest dose levels to be ‘roughened’ and have a mottled cortex. Kidneys of female rats appeared normal. Renal tubular dilatation and hypertrophy with foci of renal tubular epithelial degeneration and regeneration was observed in males and females in the high dose group and one female at the second highest dose group. Histopathological examination of weanling rats found no treatment-related lesions.

A similar, limited, 1-generation study fed diets containing 0, 150 or 1500 ppm HCBD to 18 female and 6 male Wistar rats for 4 weeks prior to mating (Harleman and Seinen, 1979). It was not clear if exposure continued through the mating period (3 weeks), and then through gestation and weaning. At the highest dose, rats showed obvious signs of toxicity (ataxia) and no conception occurred. At the 150 ppm dose level, birth weights were reduced and growth retarded but no grossly observable malformations were seen. Toxicity typical of HCBD exposure (kidney lesions and reduced body weights) were apparent in adults at both dose levels. In addition, femoral nerves showed fragmentation and demyelinization in rats at the highest dose.

VI. Derivation of Chronic Reference Exposure Level (REL)

Inhalation Chronic Reference Exposure Level

Study	Saillenfait *et al.* (1989)
Study population	Sprague-Dawley rats (24 or 25 rats per sex per group)
Exposure method	Discontinuous whole-body inhalation exposure (0, 2, 5, 10, or 15 ppm)
Critical effects	Reduced maternal weight gain
LOAEL	15 ppm
NOAEL	10 ppm
Exposure continuity	6 hours per day
Exposure duration	Days 6 through 20 of gestation
Average experimental exposure	2.5 ppm for NOAEL group (10 x 6/24)
Human equivalent concentration	2.5 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.008 ppm (8 ppb, 0.09 mg/m^3, 90 $\mu g/m^3$)

Oral Chronic Reference Exposure Level

Study	Yang *et al.*, 1989; NTP, 1991
Study population	10 mice/group/sex, 120 total.
Exposure method	Orally, in diet (0, 1, 3, 10, 30 or 100 mg HCBD/kg diet, equivalent to 0, 0.1, 0.4, 1.5, 4.9 and 16.8 mg/kg body wt-day for females and 0, 0.2, 0.5, 1.8, 4.5 and 19.2 mg/kg body wt-day for males)
Critical effects	Kidney (dose-dependent increase in renal tubular epithelial regeneration characterized by increased basophilia of the tubular cell cytoplasm, increased number of nuclei and occasional mitotic figures.)
LOAEL	0.1 mg/kg bw-day (1 mg HCBD/kg diet)
NOAEL	Not observed
Exposure continuity	Orally, in diet *ad libitum*
Exposure duration	13 weeks
Average experimental exposure	0.013 mg/kg bw-day

LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies factor	10
Cumulative uncertainty factor	1,000
Oral reference exposure	0.00001 mg/kg bw-day

Following 13-week exposure of female mice to HCBD in diet, adverse effects to the kidney tubular epithelium occurred in a dose-response fashion among all exposure groups (Yang *et al.*, 1989). Therefore, a NOAEL was not observed. However, this finding represents the most sensitive indicator of toxicity from HCBD among all the studies discussed above. Male mice were also susceptible to the nephrotoxic effects of HCBD, but at higher doses (4.5 and 19.2 mg/kg body wt-day). The average experimental exposure of the LOAEL that represents continuous HCBD exposure over an entire lifespan, as opposed to the subchronic exposure schedule used in the study (13 weeks), was calculated to be 0.013 mg/kg bw-day. Thus, a subchronic uncertainty factor was not required. An uncertainty factor of 10 was applied to arrive at an estimate of the concentration at which adverse effects might not be observed in a lifetime exposure study. Applying additional uncertainty factors of 10 each to account for interspecies differences and to account for any increased susceptibility of sensitive human populations, an oral reference exposure level (REL) of 0.00001 mg/kg bw-day (0.01 μg/kg body wt-day) was obtained.

The REL determined from the Yang *et al.* (1989) study is consistent with a chronic REL determined from the human occupational study by Driscoll *et al.* (1992). This study indicates that small disturbances in liver function occurs with long-term (average, 8.9 years) exposure to HCBD, resulting in a LOAEL of 5 ppb. Extrapolation from the workplace LOAEL to a continuous time-weighted average exposure results in an average experimental exposure of approximately 1 ppb. Applying uncertainty factors of 10 each to account for sensitive humans and to extrapolate from the LOAEL to a NOAEL results in REL of 0.01 ppb. Standard organ function tests for the kidney, the primary target organ, were not performed. Therefore, the chronic REL is based on the slightly lower values determined by Yang *et al.* (1989) in experimental animals.

All the major chronic and subchronic studies are consistent with the major toxicological findings produced (kidney lesions, increased kidney weight, reduced body weights) following HCBD exposure. At higher doses, other adverse effects (hepatotoxicity, femoral nerve demyelinization) were observed in some studies. However, nearly all the studies found that the females of both rats and mice are more sensitive to the chronic effects of HCBD. Furthermore, female mice appear more sensitive to the kidney effects of HCBD than female rats, resulting in a chronic REL based on kidney lesions observed in female mice.

Weaknesses of the database for HCBD include the lack of human exposure data and the lack of long-term inhalation studies. Studies showing clear adverse effects in humans due exclusively to HCBD has not been found in the literature. The best epidemiological data may eventually come from other countries which use HCBD as a pesticide. Even though HCBD exhibits relatively

poor volatility, inhalation can still be an important route of exposure. A subchronic inhalation study would strengthen the database for HCBD. Due to the potent nephrotoxicity of this chemical and the varying degree of toxicity observed between species and the sexes, an additional subchronic study in a non-rodent species would also enhance the HCBD toxicity database.

VII. References

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CHRONIC TOXICITY SUMMARY

HEXACHLOROBENZENE

(HCB; perchlorobenzene; pentachlorophenyl chloride; phenyl perchloryl)

CAS Registry Number: 118-74-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**3 μg/m^3**
Oral reference exposure level	0.0008 mg/kg-day
Critical effect(s)	Liver (hepatic centrilobular basophilic chromogenesis in rats)
Hazard index target(s)	Alimentary system

II. Chemical and Physical Properties (HSDB, 1995)

Molecular formula:	C_6Cl_6
Molecular Weight:	284.80
Description:	White crystalline solid, white needles from benzene.
Vapor Pressure:	1.09 x 10^{-5} mm Hg at 20°C
Solubility:	Practically insoluble in water (0.006 mg/L H_2O at 20°C). Soluble in benzene, chloroform and ether. Sparingly soluble in cold alcohol.
Conversion factor:	11.65 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

HCB has not been produced as a commercial product in the U.S. since the 1970s. However, HCB is produced as a by-product or impurity in the manufacture of chlorinated solvents, other chlorinated compounds, and several pesticides, including pentachloronitrobenzene, chlorothalonil, dacthal and picloram (ATSDR, 1990). HCB can also be produced during combustion processes such as incineration of municipal wastes. Currently, there are no commercial uses for HCB in the U.S. However, it was used as a pesticide until 1985. The chemical was also used in the production of pyrotechnic and ordnance materials for the military and in the production of synthetic rubber. HCB does not occur naturally. Exposure to HCB can occur through contact with HCB-contaminated soil, dust particles, or industrial releases into the environment. Under ordinary conditions, the evaporation of HCB into the air is not significant. Because of its low water solubility, HCB is usually not present in drinking water.

IV. Effects of Human Exposure

In humans, inhalation is most likely a minor route of exposure to HCB due to its low vapor pressure. Exposure to HCB by inhalation was estimated to average 2 ng/m^3 of air breathed. This is approximately 1000 times less than exposure estimated by ingestion (Burton and Bennett, 1987). In a study of 50 industrial workers, HCB blood levels were strongly correlated with years worked in the chlorinated solvents industry, but poorly correlated with environmental exposure measurements (Currier *et al.*, 1980). No adverse effect was associated with worker exposure to HCB.

The principal epidemiological study of chronic HCB toxicity occurred in Turkey from 1955 to 1959 and involved the accidental consumption of wheat treated with HCB (used as a fungicide) (Cam and Nigogosyan, 1963). The primary clinical sign was porphyria cutanea tarda: sunlight-sensitive skin lesions characterized by bullae, milia, extensive scarring, loss of elasticity, atrophy, hyperpigmentation and alopecia. Abnormal levels of porphyrin precursors found in the blood, urine and feces suggest that HCB disturbed the porphyrin metabolism of the liver which caused histopathologic changes in the organ. HCB also produced neurological effects, including muscle weakness, cogwheel rigidity and sensory shading. Perhaps of greatest concern was that nearly all children born to porphyric mothers died by age 2 of pembe yara (pink sore disease), thought to be related to transplacental and maternal milk HCB levels (Peters *et al.*, 1982). These children developed pink sores which resulted in death due to cardiorespiratory failure and sometimes tremors and convulsive episodes. A follow-up of HCB-poisoned patients 25 years later revealed that over 50% still exhibited many of the dermatological and neurological symptoms (Peters *et al.*, 1982; Cripps *et al.*, 1984). In addition, enlarged thyroid was observed in 60% of the women and 27% of the men. Patients who exhibited acute porphyria before puberty developed shortened hands, swelling and spindling of the fingers and painless arthritis 20-25 years later. Lactation specimens of female patients 20-25 years following exposure to HCB showed that high levels of the chemical (up to 3.12 ppm) were still present, but infant offspring appeared normal.

Further confirmation of HCB's environmental persistence and bioaccumulation in humans is evident in the study by Stanley (1986). HCB was found in 98 of 100 human adipose tissue samples from all regions of the U.S. at levels ranging from 1 to 9 ng/g.

V. Effects of Animal Exposure

Absorption of orally administered HCB is dependent on the solvent vehicle used. In rats, 80% of HCB was absorbed with olive oil as the vehicle, whereas only 6% of HCB was absorbed when administered in an aqueous suspension (Koss and Koransky, 1975). The lymphatic system has been shown to play an important role in HCB uptake from the intestine (Iatropoulos *et al.*, 1975). Following absorption by the lymphatic system, HCB can be deposited in the adipose tissue, bypassing the portal circulation. Due to its lipophilic nature, HCB is preferentially distributed to the adipose tissue and other tissues with high lipid content (ATSDR, 1990). HCB is concentrated in the milk and is readily transferred to infants during nursing. In lactating female

rhesus monkeys given HCB by gavage for 60 days, the concentration of HCB was 17 times higher in milk than that observed in serum (Bailey *et al.* 1980). Nursing infants had blood levels 2 to 5 times higher than that of their mothers. Once absorbed, some HCB is slowly metabolized to less chlorinated benzenes, chlorinated phenols, other minor metabolites, and glucuronide and glutathione conjugates (ATSDR, 1990). HCB can also induce various hepatic enzymes. In Rhesus monkeys (Rozman *et al.*, 1977) and rats (Mehendale *et al.*, 1975), HCB was excreted primarily in the feces with 99-100% of the chemical remaining intact. Approximately 75% of radiolabelled HCB excreted in urine was either pentachlorophenol or pentachlorobenzene (Rozman *et al.*, 1977). In rats, HCB exhibits multi-compartmental pharmacokinetics with a half-life of up to 12 months from poorly perfused tissues, such as fat (Koss *et al.*, 1983). At least 70 to 80% of a single dose of HCB is retained in the rat for 7 days (Mehendale *et al.*, 1975)

No studies were located regarding the pharmacokinetics or toxicity of HCB following inhalation in animals.

Despite the abundance of subchronic feeding studies that investigated noncancer effects of HCB, only one long-term study has been performed in which noncancer chronic effects were well documented. This report was a two-generation feeding study in which the f_1 generation was placed on a diet containing HCB for their lifespan (Arnold *et al.*, 1985). Forty to 66 Sprague-Dawley rats/sex/group were fed from weaning on diets containing 0, 0.32, 1.6, 8.0 or 40.0 ppm HCB. At three months, the F_0 rats were bred and 50 pups (F_1) of each sex were fed their respective parents' diets. Thus, the F1 rats were exposed to HCB, beginning in utero, for their entire lifespan (130 weeks). There were no treatment-related effects on growth, mortality, feed consumption or hematological parameters in either generation. However, pup viability was significantly reduced in the 40 ppm group. Increased heart and liver weights were found in F_0 males of the 8.0 and 40 ppm group, but no values were given. Histopathology of the F_1 generation revealed a significant increase in centrilobular basophilic chromogenesis of the liver in the 8 and 40 ppm groups. An increased incidence of severe chronic nephrosis was observed in males of the 40 ppm group, but many of the controls also had this kidney lesion. No treatment-related alopecia or dermal changes were noted in HCB-exposed rats. Urinary porphyrin excretion and liver porphyrin levels were not done.

The most comprehensive subchronic HCB feeding study was performed by Kuiper-Goodman *et al.* (1977). Seventy rats/group/sex were fed the equivalent of 0, 0.5, 2.0, 8.0 or 32.0 mg of HCB/kg body weight for up to 15 weeks. By 15 weeks, decreased average body weight (13%) in male rats and significantly increased mortality in female rats were noted at the highest dose level. Clinical signs of toxicity seen at this dose level included intention tremors, irritability and multiple alopecia areas with accompanying scabbing. Relative kidney and spleen weights at the highest dose level and relative liver weights at the two highest dose levels were significantly greater for most of the treatment period. By 12 weeks, female rats in the two highest dose groups had increased porphyrin levels whereas males in the high dose group had only slightly elevated porphyrin levels toward the end of the treatment period. Porphyrin levels remained high in female rats up to 33 weeks following cessation of HCB exposure. Elevated levels of porphyrins even appeared occasionally in the lower two dosage groups during the post-exposure period. Gross pathological and histopathological changes were confined to the liver and spleen. At the

two highest dose levels, livers were enlarged and exhibited hepatocellular hypertrophy. Centrilobular lesions were present but were also seen in livers of the control group as well. Under electron microscopy however, hepatocytes of female rats with advanced porphyria exhibited extensive vacuolation containing dark inclusion bodies. Congestive splenomegaly was observed in many female rats at the highest dose level by 12 weeks. Overall, females were more sensitive than males to the toxic effects of HCB.

In a twelve month HCB feeding study, 6 beagle dogs/group/sex were given 0, 1, 10, 100 or 1000 mg HCB/day in gelatin capsules (Gralla *et al.*, 1977). A transient increase in mortality, anorexia and weight loss was observed among dogs in the two highest dose groups the first three months. Blood analysis revealed neutrophilia in some dogs at the two highest dose levels. Treatment-related effects appeared to be confined to the abdomen and included serositis, necrosis, fibrosis and steatitis of the omentum. Nodular hyperplasia of gastric lymphoid tissue was found in all treated dogs but was also present in some controls. Hepatic porphyria was not observed.

In a 90-day toxicity study in SPF pigs (5 males/group) given 0, 0.05, 0.5, 5.0 or 50 mg/kg/day of HCB in diet, a small but significant increase in coproporphyrin excretion was observed at the three highest doses (Den Tonkelaar *et al.*, 1978). However, this effect was seen only at 8 weeks and other clinical signs of porphyria (fluorescence of liver under UV light) were absent. Pigs given the highest dose of HCB died by 10 weeks. Induction of microsomal enzymes occurred in the 5.0 and 0.5 mg/kg groups. Neutrophilia was seen at the two highest doses. Significant increases in liver, kidney and thyroid weight were observed in the 5.0 mg/kg group. Hepatocytes showed centrilobular hypertrophy at this dose level.

In four female rhesus monkeys exposed to HCB (one monkey each at 8, 32, 64 mg/kg body wt, and 2 at 128 mg/kg body wt) by oral gavage for 60 days, dose-dependent degenerative changes were noted in the thymic cortex and ovaries (Iatropoulos *et al.*, 1976). Hepatocellular degenerative changes characteristic of porphyria tarda, vacuoles and granular deposits, occurred in all HCB-treated monkeys. However, urinary porphyrin excretion and liver porphyrin levels were not examined. Kidney degenerative changes were observed but were apparently not dose-dependent. No neurological alterations were observed. No cutaneous alterations were observed either, but it was not indicated in the report whether monkeys were exposed to direct sunlight.

Other subchronic studies of HCB have investigated specific adverse effects, such as immune system effects and hyperparathyroidism. Female Wistar rats fed a diet with 150 and 300 mg HCB/kg for 13 weeks had elevated spleen and popliteal lymph node weights and elevated IgM, IgM-anti-ssDNA, and IgM-anti-BrMRBC serum levels, but unchanged IgG levels (Schielen *et al.*, 1995). HCB also caused a dose-dependent increase of the incidence, but not the severity, of skin lesions. Male Fischer 344 rats fed a diet containing 0, 0.1, 1.0, 10.0 or 25.0 mg HCB/kg for up to 15 weeks had significantly elevated liver and kidney weight and parathyroid hormone at the two higher dose levels (Andrews *et al.*, 1989). Serum alkaline phosphatase was significantly decreased at the two higher dose levels. 1,25-Dihydroxyvitamin D_3 was measured at 5 weeks and was significantly elevated in the three higher dose levels. Altered levels of these hormones and enzymes may be linked to interference of calcium homeostasis, and consequently lead to osteosclerosis.

In a 4-generation reproduction study, pregnancy, viability and lactation indices were measured in Sprague-Dawley rats fed 0, 10, 20, 40, 80, 160, 320 and 640 ppm HCB beginning at weaning (Grant *et al.*, 1977). When F_0 rats reached 100 days of age, 1 male was caged with 2 females for 1 week, and then the males were rotated for a second one-week mating period. Increased mortality was observed in F_0 generation females at the two highest dietary concentrations. For the F_1 generations, the viability index was zero at the two highest concentrations and 55% in the 160 ppm group. The lactation index, the most sensitive index measured, was about 30% in the F_1 generations fed 160 ppm and zero in the F_2 generations. A dietary level of 80 ppm HCB regularly decreased the body weight of 5- and 24-day-old pups by 10% or greater. Liver weights of F_3 pups from dams fed 40 ppm HCB increased approximately 10%. No gross abnormalities were observed in any of the pups.

Further evidence of the high risk to nursing infants when mothers are exposed to HCB was shown in a study by Kitchin *et al.* (1982). Female Sprague-Dawley rats were fed either 0, 60, 80, 100, 120 or 140 ppm HCB in diet for at least 96 days before beginning of gestation and continued throughout the experiment. Twenty-one-day mortality was 10% greater in offspring of dams fed 60 ppm HCB and more than 80% greater in offspring of dams fed 120 ppm HCB, when compared to 21-day mortality of the control group. Clinical signs of maternal toxicity were not observed and fertility and fecundity were unaffected.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Arnold *et al.*, 1985
Study population	40-66 rats/group/sex (500 total) in F_0 generation; 50 rats/sex/group (500 total) in F_1 generation.
Exposure method	Dissolved in corn oil, mixed in diet (0, 0.32, 1.6, 8.0, and 40.0 ppm).
Critical effects	Hepatic centrilobular basophilic chromogenesis (F_1 generation)
LOAEL	8.0 ppm in diet (0.4 mg/kg-day)
NOAEL	1.6 ppm in diet (0.08 mg/kg-day)
Exposure continuity	Orally, in diet *ad libitum*
Exposure duration	F_0 generation, approximately 18 weeks; F_1 generation, 130 weeks
Average experimental exposure	0.08 mg/kg-day
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies factor	10
Cumulative uncertainty factor	100
Oral reference exposure	0.0008 mg/kg-day (U.S. EPA RfD)
Inhalation conversion factor	3,500 $\mu g/m^3$ per mg/kg-day
Inhalation reference exposure level	3 $\mu g/m^3$ (0.2 ppb)

The oral HCB exposure study by Arnold *et al.* (1985), on which the chronic REL is based, is the only chronic study in which noncancer adverse effects are well documented. The results also produce the lowest LOAEL and NOAEL. Lifetime exposure of rats, beginning in utero, to hexachlorobenzene resulted in adverse effects to the liver at the second highest dose level of 8.0 ppm HCB in diet. Adverse effects were not seen at the two lowest dose levels of 0.32 or 1.6 ppm HCB in diet. Since average food consumption values were not included in the published study, it was estimated that rats eat approximately 5% of their body weight daily. Therefore, the LOAEL of 8.0 ppm HCB in diet is equivalent to 0.4 mg/kg-day and the NOAEL of 1.6 ppm HCB in diet is equivalent to 0.08 mg/kg-day. Assuming uncertainty factors of 100 to account for interspecies differences and the increased susceptibility of sensitive human populations, an oral reference exposure level of 0.0008 mg/kg-day is estimated. This value is equivalent to an inhalation REL of 3 $\mu g/m^3$ for humans (assuming a daily respiration rate of 20 m^3 of air and an average body weight of 70 kg).

The adverse effects observed in chronic and subchronic toxicity studies with experimental animals, particularly in rats, are similar to those seen in humans. Both humans and rats develop porphyria with long-term exposure to HCB; in fact, the females of both humans and rats appear more susceptible to this adverse effect. The primary target organ for noncancer effects is the liver. Nursing infants appear to be particularly sensitive to HCB toxicity if the mother is chronically exposed to HCB. HCB is concentrated in the milk of humans as well as in experimental animals.

In the study by Arnold *et al.* (1985), a strong dose-response effect is observed regarding centrilobular basophilic chromogenesis. A dose-response effect was also seen regarding peliosis (hemorrhages in the skin) in females only and peribiliary lymphocytosis and severe chronic nephrosis in males only. With the exception of centrilobular basophilic chromogenesis, and possibly peliosis, these effects may not be treatment-related because many of the controls also had these adverse effects. Moreover, the severe chronic nephrosis observed in male rats exposed to HCB has been reported to be HCB-induced male rat specific nephropathy (Bouthillier *et al.*, 1991), and therefore not relevant to humans.

Weaknesses of the database for HCB include the lack of lifetime toxicity studies in species other than rats. Long-term studies have been undertaken in mice and hamsters but were primarily interested in carcinogenesis. An adequate investigation of noncancer effects was not performed. A subchronic feeding study performed in pigs (Den Tonkelaar *et al.*, 1978) indicate that adverse effects occur near dose levels observed for adverse effects in the long-term rat study. A long-term study in pigs may reveal this species to be more sensitive to HCB than rats.

The strengths of the inhalation REL include the availability of chronic exposure data from a well-conducted study with histopathological analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data and the lack of chronic inhalation exposure studies.

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CHRONIC TOXICITY SUMMARY

HEXACHLOROCYCLOHEXANE (alpha-ISOMER)

(Alpha-benzene hexachloride; alpha-BHC; alpha-HCH; alpha-lindane)

CAS Registry Number: 319-84-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**20 μg/m^3**
Oral reference exposure level	**0.005 mg/kg body wt-day**
Critical effect(s)	Hepatocyte hypertrophy, hepatic cell atrophy and fatty degeneration in rats
Hazard index target(s)	Alimentary system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula:	$C_6H_6Cl_6$
Molecular weight	290.85
Description	Technical grade hexachlorocyclohexane is a brownish to white crystalline solid with a phosgene-like odor.
Vapor pressure	0.02 mm Hg at 20°C
Solubility	practically insoluble in water (10 ppm at 25°C); soluble in alcohol.
Conversion factor	11.90 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

Alpha-hexachlorocyclohexane (alpha-HCH) is a byproduct (<1%) in the synthesis of the insecticide lindane (gamma-hexachlorocyclohexane) (HSDB, 1995). Alpha-HCH has only marginal activity against insects. Technical grade benzene hexachloride (BHC) contains 65-70% alpha-HCH and may still be used as an insecticide in some developing countries. BHC is no longer produced or sold for domestic use in the U.S. (Farm Chemicals Handbook, 1995). The compound is also a by-product of chlorinations to produce chlorobenzene, dichlorobenzene and tetrachloroethylene. Small amounts of alpha-HCH may result from the isomerization of lindane upon exposure to sunlight. Release of alpha-HCH to the environment probably occurs mainly from the use of technical hexachlorocyclohexane as a pesticide.

IV. Effects of Human Exposure

The most probable route of alpha-HCH exposure in humans is ingestion of food containing the pesticide. A lesser degree of exposure may result from oral ingestion of drinking water. It should be emphasized, however, that use of technical grade BHC (which contains alpha-HCH) has been discontinued in the U.S. for some time. True chronic poisoning, distinct from symptoms of acute exposure, have not been described in man.

In India, serum hexachlorocyclohexane residues were studied in 64 employees of a pesticide manufacturing plant (Nigam *et al.*, 1986). Duration of exposure ranged from 0-30 years. Total HCH residue levels and the levels of the alpha, beta and gamma isomers in the exposed group were significantly higher than the respective values in the controls. The mean serum HCH level (0.604 ppm) in this group was 12 times that of the controls. Many of the workers showed acute signs of HCH intoxication (i.e. paresthesia of the face and extremities, headache, giddiness, etc.).

Another study in India investigated an outbreak of poisoning due to the consumption of HCH (65-70% alpha-HCH) admixed food stuffs, in some cases for as long as 2 years (Ramachandran *et al.*, 1982). Acute symptoms of HCH intoxication were seen but chronic symptoms were not reported. Blood levels of HCH had decreased only 20 to 40 percent six months after cessation of exposure to contaminated food stuffs. This study confirmed the persistence and slow elimination of the residues from the body.

V. Effects of Animal Exposure

In rats, when radiolabelled alpha-HCH is injected intraperitoneally, 80% of the total radioactivity is excreted in the urine and 20% in the feces (Koransky *et al.*, 1964). In another rat study, animals were fed 800 mg/kg alpha-HCH in the diet for 20 months. Following cessation of dietary exposure, alpha-HCH disappeared from fat stores within three weeks (Davidow and Frawley, 1951). Conjugated 2,4,6,-trichlorophenol is the major urinary metabolite (IARC, 1979). The metabolite 2,4,5,-trichlorophenol has also been identified in urine.

In a 2-year feeding study (Fitzhugh *et al.*, 1950), 10 Wistar rats/sex/group were exposed to 0, 10, 50, 100, or 800 ppm alpha-HCH in their diet. These diet concentrations are equivalent to 0, 0.5, 2.5, 5, and 40 mg/kg body wt-day of alpha-HCH (Gold *et al.*, 1984). Weight gain was reduced (12-15%) and mortality was significantly higher in the high dose group. Kidney and spleen organ weight was not affected but liver weight was significantly higher in the 50 ppm group and above. Very slight liver damage (hepatocellular hypertrophy, hepatic cell atrophy, fatty degeneration and focal necrosis) was seen at 50 ppm. Damage to kidney was less pronounced; a small amount of tubular dilation and/or atrophy was seen at 800 ppm.

In a 24 week feeding study (Nagasaki *et al.*, 1975), 500 ppm alpha-HCH in the diet increased liver weight and caused hepatocellular hypertrophy in rats, mice and hamsters. Mice appeared to be more susceptible to the tumorigenic activity of alpha-HCH than rats and hamsters. No other non-neoplastic histopathological findings were described.

Hepatocellular hypertrophy by 16 weeks was also a common finding in mice fed 500 ppm alpha-HCH in a 36 week feeding study (Ito *et al.*, 1973). By 8-12 weeks following cessation of alpha-HCH in diet, liver cell hypertrophy had completely disappeared. No other specific chronic noncancer effects were described.

Other long-term alpha-HCH feeding studies are present in the literature but they are almost exclusively liver carcinogenic studies in mice. Examination of other organs and various clinical parameters for chronic noncancer effects were seldom undertaken by these researchers.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Fitzhugh *et al.*, 1950
Study population	10 rats/group/sex, 100 total
Exposure method	Ingestion, in diet (0, 10, 50, 100, or 800 ppm, equivalent to 0, 0.5, 2.5, 5 and 40 mg/kg body wt-day, respectively).
Critical effects	Liver (Hepatocyte hypertrophy, hepatic cell atrophy and fatty degeneration).
LOAEL	2.5 mg/kg body wt-day
NOAEL	0.5 mg/kg body wt-day
Exposure continuity	Daily *ad libitum* ingestion in diet
Exposure duration	2 years
Average experimental exposure	0.5 mg/kg body wt-day
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Oral reference exposure	0.005 mg/kg body wt-day
Inhalation conversion factor	3.5 mg/m^3 per mg/kg bw-day
Inhalation reference exposure level	0.001 ppm (1 ppb, 0.02 mg/m^3, 20 $\mu g/m^3$)

Nearly all the research conducted with alpha-HCH was concerned with its potential carcinogenic effects on a sensitive species (the mouse). In fact, Ito *et al.* (1975) presented evidence that among the four main HCH isomers (α-, β-, δ-, and γ-isomers), only alpha-HCH had carcinogenic activity in male strain dd mice. Only one true chronic noncancer toxicity study with alpha-HCH was present in the literature (Fitzhugh *et al.*, 1950). While this study did not describe the pathology in detail and may have had too few animals in the histopathological exam, it did run a similar chronic toxicity study with the gamma isomer, lindane, alongside the alpha-HCH study. The chronic toxicity of lindane is well known (EHC 124, 1991). With respect to the relative potency of these two HCH isomers on liver and kidney injury, alpha-HCH appears to be slightly more toxic to the liver and slightly less toxic to the kidney than lindane. The types of injuries

produced in these two organs by alpha-HCH and lindane, probably the most sensitive indicators of chronic injury for these chemicals, were similar. The result is that the chronic REL for alpha-HCH would probably be comparable to lindane.

A NOAEL of 10 ppm was established by Fitzhugh *et al.* (1950). This value is equivalent to 0.5 mg alpha-HCH/kg body wt-day, assuming rats eat 5% of their body weight/day (based on data in Gold *et al.*, 1984). Applying uncertainty factors of 10 each to account for interspecies differences and to account for any increased susceptibility of sensitive human populations, an oral reference exposure level (REL) of 0.005 mg/kg body wt-day was obtained. This value is, in turn, equivalent to an inhalation REL of 20 $\mu g/m^3$ (assuming a daily respiration rate of 20 m^3 of air and an average weight of 70 kg).

Weaknesses of the database for alpha-HCH include the lack of studies investigating the effects of the chemical on reproduction, development and the immune system. Another weakness is the absence of long-term inhalation studies. The alpha-HCH database would also be enhanced by the addition of a contemporary long-term toxicity study on a rodent and non-rodent species. Currently, continued exposure from new sources of alpha-HCH are unlikely since production and use of technical HCH has been discontinued in the U.S.

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CHRONIC TOXICITY SUMMARY

HEXACHLOROCYCLOHEXANE (beta-ISOMER)

(beta-benzene hexachloride; beta-BHC; beta-HCH; beta-1,2,3,4,5,6-hexachlorocyclohexane)

CAS Registry Number: 319-85-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**2 μg/m^3**
Oral reference exposure level	**0.0005 mg/kg body wt-day**
Critical effect(s)	Atrophy of the ovaries with impaired oogenesis and focal hyperplasia; decreased thymus (females) Atrophy of the testes including reduced size of seminiferous tubules, decreased number of interstitial cells and spermatogenic arrest (males)
Hazard index target(s)	Immune system; reproductive system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	$C_6H_6Cl_6$
Molecular Weight	290.85
Description	Crystals from benzene, alcohol or xylene.
Vapor Pressure	0.005 mm Hg at 20°C
Solubility	Practically insoluble in water (5 ppm); soluble in alcohol and chloroform.
Conversion factor	11.90 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

Beta-hexachlorocyclohexane (beta-HCH) is a byproduct (<1%) in the synthesis of the insecticide lindane (gamma-hexachlorocyclohexane) (HSDB, 1995). Beta-HCH has only marginal activity against insects. Technical grade benzene hexachloride, or hexachlorocyclohexane (HCH), contains 5-12% beta-HCH and may still be used as an insecticide in some developing countries. HCH is no longer produced or sold for domestic use in the U.S. (Farm Chemicals Handbook, 1995). Therefore, exposure from this chemical is probably not a major concern. However, environmental breakdown of beta-HCH is known to be slower than the other HCH isomers.

Beta-HCH is also a photolysis product of lindane. Release of beta-HCH to the environment probably occurs mainly from the use of technical hexachlorocyclohexane as a pesticide.

IV. Effects of Human Exposure

The most probable route of beta-HCH exposure in humans is ingestion of food containing the pesticide. A lesser degree of exposure may result from oral ingestion of drinking water. It should be emphasized, however, that use of technical grade HCH (which contains beta-HCH) has been discontinued in the U.S. for some time. True chronic poisoning distinct from symptoms of acute exposure have not been described in man. Residues of HCH have been found in human fat and milk due to the lipophilic nature of the HCH isomers. Although the α, β, and γ isomers were all found as residues, the α and γ isomers are more rapidly metabolized and the β isomer accounted for 90% of the total HCH isomer residue (Murphy, 1986). The β isomer is also more persistent in soil than the α and γ isomers (IARC, 1979).

Another study in India investigated an outbreak of poisoning due to the consumption of HCH (6-8% beta-HCH) admixed food stuffs, in some cases for as long as 2 years (Ramachandran *et al.*, 1982). Acute symptoms of HCH intoxication were seen but chronic symptoms were not reported. Blood levels of HCH had decreased only 20 to 40 percent six months after cessation of exposure to contaminated food stuffs. This study confirmed the persistence and slow elimination of the residues from the body. Unfortunately, the proportion of each HCH isomer was not determined. But it is quite likely that beta isomer made up a major portion of the remaining HCH residues in the affected population due to its tendency to bioaccumulate (Macholz, 1982).

Beta-HCH, as well as other organochlorine chemicals and insecticides, is known to have some estrogenic activity (Loeber and van Velsen, 1984). In women with fertility problems, chlorinated hydrocarbons, including beta-HCH, were found in higher concentrations in follicular fluid and cervical mucus from women who remained infertile compared to women who ultimately conceived (Wagner *et al.*, 1990). But this does not establish a causal role for these compounds in fertility problems.

V. Effects of Animal Exposure

Radiolabelled beta-HCH is 80% absorbed when administered orally to rats (IARC, 1979). Trichlorophenols and trichloroanisoles were the most common metabolites identified in urine, liver and kidney following administration of beta-HCH in diet of rats (IARC, 1979). Beta-HCH has a greater tendency to bioaccumulate than the other two main HCH isomers, alpha-HCH and gamma-HCH (Macholz, 1982).

In a 30-day feeding study, female B6C3F1 mice were exposed to 0, 100 or 300 mg beta-HCH/kg of diet to investigate toxic effects on the reproductive and immune systems (Corncob *et al.*, 1988). The number of animals per group was not given. Ovaries and endometrial epithelium

exhibited normal architecture and no changes were observed in body weight, lymphoid organ weight and histology or splenic cellularity. However, significant changes in several immune functions did occur in the 300 mg/kg group. Proliferation of splenocytes in response to the mitogens LPS, PHA, and Con A was decreased and T-lymphocyte-mediated cytolysis of tumor targets was decreased with a concurrent reduction in NK activity.

The most contemporary beta-HCH feeding study was performed in rats by van Velsen *et al.* (1986). Ten Wistar rats/sex/group were exposed to 0, 2, 10, 50 and 250 mg/kg beta-HCH in their diet for 13 weeks. Increased mortality and decreased weight gain were recorded in the high dose group. Males showed atrophy of the testes, which included reduced size of seminiferous tubules, decreased number of interstitial cells and spermatogenic arrest. Females showed atrophy of the ovaries with impaired oogenesis and focal hyperplasia. In the 50 mg/kg group, the weights of the thymus and testes were significantly reduced. Occasional cell necrosis was observed in the livers of the two highest dose groups. In the highest dose group, a larger degree of periportal and diffuse fat accumulation was noted. Among all dosage groups, a dose-dependent increase was noted in liver weight, centrilobular hepatocytic hypertrophy, proliferation of smooth endoplasmic reticulum and microsomal activity.

In a two year feeding study by Fitzhugh *et al.* (1950), 10 Wistar rats/sex/group were exposed to 10, 100, or 800 ppm beta-HCH in their diet. The γ and α isomers were also tested for their chronic toxicity in rats. Very slight liver damage was noted as low as 10 ppm beta-HCH in diet and was characterized as hepatocellular hypertrophy, hepatic cell atrophy, fatty degeneration and focal necrosis. The liver injury produced was similar among all the HCH-isomers tested, but beta-HCH had the highest chronic toxicity for the liver. No beta-HCH effects on testes were reported. Evaluation of female reproductive organ effects was not done.

In a two year carcinogenic study on CF1 mice given 200 ppm beta-HCH in their diet, enlarged livers were seen by 50 weeks (Thorpe and Walker, 1973). At the end of the study, many of the livers had irregular nodular surfaces and occasional yellow necrotic areas, most likely related to the development of tumors.

In studies investigating the estrogenic effects of beta-HCH, juvenile mice and rats were fed a diet containing various concentrations of beta-HCH for only five days (Loeber and van Velsen, 1984). A significant increase in uterine weight, a classical *in vivo* bioassay for estrogenic activity, was seen in both mice and rats at 50 mg/kg beta-HCH. However, in a longer term feeding assay by van Velsen *et al.* (1986), beta-HCH did not cause a dose-dependent increase in uterine weight. So it is unclear whether this estrogenic effect occurs following long-term beta-HCH exposure.

In other reproduction studies, fertility decreased and dominant lethality increased in male mice fed 500 ppm HCH (6-8% beta-HCH) for up to 8 months (Lakkad *et al.*, 1982). Testicular atrophy was seen in rats fed 1500 ppm HCH for 90 days (Shivanandappa and Krishnakumari, 1983).

VI. Derivation of Chronic Reference Exposure Level (REL*)*

Study	Van Velsen *et al.*, 1986
Study population	10 rats/group/sex; 100 total animals
Exposure method	Ingestion, in diet (0, 2, 10, 50, or 250 ppm in diet)
Critical effects	Atrophy of the ovaries with impaired oogenesis and focal hyperplasia; decreased thymus (females) Atrophy of the testes including reduced size of seminiferous tubules, decreased number of interstitial cells and spermatogenic arrest (males)
LOAEL	50 ppm in diet (approximately 2.5 mg/kg body wt-day)
NOAEL	10 ppm in diet (approximately 0.5 mg/kg body wt-day)
Exposure continuity	Daily *ad libitum* ingestion in diet
Exposure duration	13 weeks
Average experimental exposure	0.5 mg/kg body wt-day
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	10
Intraspecies factor	10
Cumulative uncertainty factor	1,000
Oral reference exposure level	0.0005 mg/kg body wt-day
Inhalation conversion factor	3.5 mg/m^3 per mg/kg bw-day
Inhalation reference exposure level	0.0002 ppm (0.2 ppb, 0.002 mg/m^3, 2 $\mu g/m^3$)

A recent long-term oral exposure study has not been performed with beta-HCH. However, a comprehensive 13-week feeding study in rats was published in 1986 (van Velsen *et al.*, 1986). Minor liver effects were seen at the two lowest doses, 2 and 10 mg/kg diet. This included slightly increased liver weight and activities of liver enzymes. Regarding chronic noncancer toxicity, adverse effects by beta-HCH occurred in lymphoid and endocrine systems. At 250 mg/kg diet, testicular atrophy was present in the males and ovarian atrophy was present in the females. At 50 mg/kg diet, thymus and testes organs were affected in the form of decreased weight. Therefore, the LOAEL for beta-HCH is 50 mg/kg diet and the NOAEL is 10 mg/kg diet. Since average food consumption values were not included in the published study, it was estimated that rats eat approximately 5% of their body weight daily. Therefore, the LOAEL of 50 mg/kg beta-HCH in diet is equivalent to 2.5 mg/kg body wt-day and the NOAEL of 10 mg/kg beta-HCH in diet is equivalent to 0.5 mg/kg body wt-day. An uncertainty factor of 10 was applied to the NOAEL to extrapolate from subchronic to lifetime exposure to beta-HCH. Applying additional uncertainty factors of 10 each to account for interspecies differences and to account for any increased susceptibility of sensitive human populations, an oral reference exposure level of 0.0005 mg/kg body wt-day is obtained. This value is equivalent to an inhalation REL of 0.002 mg/m^3 (2 $\mu g/m^3$) for humans (assuming a daily respiration rate of 20 m^3 of air and an average body weight of 70 kg).

In the chronic feeding study by Fitzhugh *et al.* (1950), very slight liver damage was noted at 10 mg/kg diet. Using this finding as the basis for the oral REL for beta-HCH would result in a lower LOAEL and NOAEL. But since this injury was not well described and the number of animals per group (10/group/sex) was quite low for a chronic study, a NOAEL based on the van Velsen study is more appropriate.

Other studies with beta-HCH or technical grade HCH have noted adverse effects on reproductive organs (Loeber and van Velsen, 1984; Lakkad *et al.*, 1981; Shivanandappa and Krishnakumari, 1983). Epidemiologic studies have also noted possible connections between organochlorine body concentrations, including beta-HCH, and reproductive effects (Wagner *et al.*, 1990). However, this does not establish a causal role for these compounds in human fertility problems.

Weaknesses of the van Velsen *et al.* (1986) study include an *n* of only 10 for each exposure group. An *n* of 30 - 40 animals per exposure group would improve statistical measurements and provide a safeguard against possible high mortality rates. Use of additional experimental animal species in feeding studies would also enhance the data base for beta-HCH. Due to the reproductive effects of beta-HCH on mammalian species, a multi-generation study would be an important addition for the beta-HCH chronic toxicity data base.

VII. References

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CHRONIC TOXICITY SUMMARY

HEXACHLOROCYCLOHEXANE (gamma-ISOMER)

(Lindane; γ-benzene hexachloride; γ-HCH; γ-BHC; 1,2,3,4,5,6 hexachlorocyclobenzene, gamma isomer)

CAS Registry Number: 58-89-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.3 μg/m^3**
Oral reference exposure level	**0.0003 mg/kg body wt-day**
Critical effect(s)	Liver hypertrophy and kidney tubular degeneration, hyaline droplets, tubular distention, interstitial nephritis and basophilic tubules in rats
Hazard index target(s)	Alimentary system; kidney

II. Chemical Property Summary (HSDB 1995)

Molecular formula	$C_6H_6Cl_6$
Molecular Weight	290.85
Description	White to yellow, crystalline powder with a slight, musty odor.
Vapor Pressure	9.4 x 10^{-6} mm Hg at 20°C
Solubility	Practically insoluble in water (7.3 ppm at 25°C); slightly soluble in mineral oils; soluble in acetone, chloroform, benzene and other organic solvents.
Conversion factor	11.90 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

γ-Hexachlorocyclohexane, commonly known as lindane, is a broad-spectrum organochlorine insecticide/acaracide registered for control of insects and other invertebrates on a wide variety of sites (HSDB 1995). Lindane is currently used for agricultural crop seed treatments, hardwood logs and lumber, forest trees, pecans, commercial ornamentals, livestock and pets, and existing structures. As of 1992, lindane was still used topically in man as an ectoparasiticide, ovicide and a scabicide to control certain parasitic organisms (PDR, 1992). The γ isomer is the effective insecticide among the eight well-described stereoisomers of hexachlorocyclohexane. Release of

lindane to the environment occurs during its use as an insecticide. Lindane is more volatile than most organochlorine insecticides. About 20% of an applied dose to crops volatilizes after 96 hours at 16°C (Starr and Johnson, 1968). Evaporation of lindane is dependent on temperature and humidity.

IV. Effects of Human Exposure

Greater than 90% of lindane exposure in humans (in industrialized countries) is due to oral ingestion of food containing the pesticide. A lesser degree of exposure may result from oral ingestion of drinking water. Because it has a half-life for elimination of over 20 hours, repeated exposure to lindane results in accumulation of the substance or its metabolites in the body. Due to its lipophilicity, lindane is preferentially deposited in the fat stores of the body (log octanol/water partition coefficient = log Kow 3.72). In metabolism studies of 21 workers producing lindane, the pesticide was excreted in urine as a number of mono-, di-, tri-, and tetrachlorophenols (Angerer *et al.*, 1983). Breast milk is also a major route of elimination in women.

Cirrhosis and chronic hepatitis were observed in liver biopsies from 8 workers heavily exposed to lindane, DDT or both for periods ranging from 5-13 years (Schuttman, 1968). Other cases possibly linked to chronic lindane exposure have been reported. Effects noted included blood changes such as decreased leukocytes (Brassow *et al.*, 1981), pulmonary fibrosis (with mixed exposures) (Barthel, 1974), EEG abnormalities (Czegledi-Janko and Aver, 1970), ECG abnormalities (Srivastava *et al.*, 1995), sex hormone changes (Tomczak *et al.*, 1981), signs of kidney dysfunction (Loganovskii, 1971), and one case of leukemia following aplastic anemia with mixed exposure to DDT (Hoshizaki *et al.*, 1969).

V. Effects of Animal Exposure

In mice, urinary metabolites of a single intraperitoneal injection of lindane accounted for 57% of the dose (IARC Monographs, 1979). The metabolites consisted mainly of glucuronide and sulfate conjugates of 2,4,6-trichlorophenol and 2,4-dichlorophenol. In rats, intraperitoneal administration of lindane yielded trichlorophenols in urine, free or as conjugates with glucuronic and/or sulfuric acid. Lindane given orally to female rats during gestation can readily cross the placenta once in the bloodstream and deposit in fetuses (Khanna *et al.*, 1991).

Effects of subchronic lindane exposure was evaluated by Zoecon Corporation (as cited in EHC 124, 1991). Lindane was administered at dietary levels of 0, 0.2, 0.8, 4, 20, or 100 ppm to male and female Wistar KFM-Han rats for 12 weeks. Rats receiving 20 and 100 ppm lindane were observed to have greater-than-control incidence of liver hypertrophy, kidney tubular degeneration, hyaline droplets, tubular distention, interstitial nephritis, and basophilic tubules. No treatment-related effects were noted with respect to mortality, hematology, or clinical chemistry, although rats fed 100 ppm in diet gained 8.4-14.9% less weight than controls. After a

recovery period, only nephritis and basophilic tubules were still present in rats that had received 100 ppm in diet. No effect was observed with doses of 4 ppm and below.

In a 2-year feeding study (Fitzhugh *et al.*, 1950), 10 Wistar rats/sex/group were exposed to 5, 10, 50, 100, 400, 800, or 1600 ppm lindane in their diet. Slight liver and kidney damage and increased liver weights were noted at the 100 ppm level. Similar results were attained in another lifetime feeding study in rats by Truhaut (1954). A dose of 25 mg/kg in diet had no effect while 50 mg/kg in diet resulted in hepatocellular hypertrophy and slight fatty hepatocyte degeneration.

In another 2-year bioassay (Rivett *et al.*, 1978), four beagle dogs/sex/group were administered 0, 25, 50, or 100 ppm lindane in the diet. The only treatment-related effects noted were in the 100 ppm group and consisted of increased serum alkaline phosphatase and enlarged dark friable livers.

In mice, the findings following long-term feeding studies with lindane were largely confined to increased tumor incidence, mainly in the liver (Thorpe and Walker, 1973; Weisse and Herbst, 1977). Chronic noncancer pathology, when investigated, was rarely found in mice.

In a three-generation study, CD rats were administered lindane at concentrations of 0, 25, 50 or 100 mg/kg-body wt in diet continuously (Palmer *et al.*, 1978a). No reproductive or developmental effects were seen. The only significant finding was that liver weights of young F3 generation rats were increased in the lindane group. Examination of the liver showed enlarged hepatocytes and vacuolization in animals treated with 50 and 100 mg/kg body wt-day in diet.

In other reproduction studies, no evidence of any teratogenic or embryotoxic effect occurred when lindane was administered during pregnancy to groups of New Zealand White rabbits (0, 5, 10 or 15 mg/kg body wt) from days 6 to 18 , or to groups of CFY rats (0, 5, 10 or 15 mg/kg body wt) from days 6 to 16 (Palmer *et al.*, 1978b). However, lindane administered by gavage to female rabbits three times a week for 12-15 weeks resulted in significantly lower ovulation rates (Lindenau *et al.*, 1994). But no significant effect was observed due to lindane regarding fertilization rate and pre- and post-implantation losses (Seiler *et al.*, 1994). Lindane given orally to female Swiss mice during gestation resulted in a variety of adverse effects including failure of implantation, fetal resorption, incomplete development and fetal death (Sircar and Lahiri, 1989). The dose was 50% of the LD_{50} (about 43 mg/kg-body wt) and given over a specified period as per experimental schedule. Administration of estrogen and progesterone during gestation abolished the adverse effects on reproduction produced by lindane.

Lindane has also been shown to have adverse effects on the male reproductive system. Chowdhury and Gautam (1994) saw a significant decline in rat testicular weight following daily intraperitoneal injections of 4 mg/kg (averaged daily dose 0.34 mg/kg) lindane for 45 days. Cellular degeneration in Leydig cells was seen at 8 mg/kg. However, testicular atrophy was only seen in rats fed 1500 ppm technical grade HCH for 90 days (Shivanandappa and Krishnakumari, 1983). Lower oral doses (100 and 750 ppm) had no effect on testes.

A few inhalation studies have been performed with lindane. In a 14-week study, mice were exposed to 0, 0.3, 1.0 or 5-10 mg/m^3 lindane (EHC 124, 1991). Exposures were for 6 hr/day, 5 days/week. No effect was seen in males, but 5 and 10 mg/m^3 were toxic to females. Specific pathological findings were not reported. In rats, inhalation of lindane at nominal concentrations of 0, 0.02, 0.12, 0.6 or 4.5 mg/m^3 was performed daily for 6 hr/day for three months (Oldiges *et al.*, 1980). Increased kidney weight and cloudy swelling of tubular epithelium were reported at 0.6 and 4.5 mg/m^3 of lindane. Increased P-450 activity was observed at 4.5 mg/m^3 lindane. No other changes were seen.

In studies on specific organ systems, Saha and Benerjee (1993) indicated that chronic lindane exposure may have a deleterious effect on humoral and cell-mediated immune responses. Wistar rats were fed a diet containing 0, 5, 20, or 30 ppm lindane for 22 weeks. The number of animals per group was not described. At 20 ppm, there was a decreased antibody response to tetanus toxoid, decreased serum immunoglobulin concentrations following tetanus toxoid stimulation, and inhibition of leukocyte and macrophage migration. Lahiri and Sircar (1991) reported adverse affects on adrenocortical function of female mice given approximately 46 mg lindane/kg-body wt orally for 4-6 weeks. Adrenal weight and glucocorticoid content were reduced significantly. Histopathological analysis showed marked atrophy of the cortical region.

VI. Derivation of Chronic Reference Exposure Levels (REL)

Derivation of Inhalation Reference Exposure Level

Study	Oldiges *et al.* (1980)
Study population	Rats
Exposure method	Inhalation
Critical effects	Increased kidney weight and cloudy swelling of tubular epithelium
LOAEL	0.6 mg/m^3
NOAEL	0.12 mg/m^3
Exposure continuity	6 hr/day
Exposure duration	3 months
Average experimental exposure	0.03 mg/m^3 (0.12 x 6/24)
Human equivalent concentration	0.03 mg/m^3 (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.0003 mg/m^3 (0.3 μg/m^3, 0.00003 ppm, 0.03 ppb)

Derivation of Oral Reference Exposure Level

Study	Zoecon Corporation, 1983 (as cited in EHC, 1991)
Study population	20 rats/sex/group; 240 total animals
Exposure method	Ingestion, in diet
Critical effects	Liver hypertrophy, kidney tubular degeneration and distention, interstitial nephritis, basophilic tubules
LOAEL	20 ppm in diet (about 1.45 mg/kg body wt-day)
NOAEL	4 ppm in diet (males, 0.29 mg/kg body wt-day; females, 0.33 mg/kg body wt-day)
Exposure continuity	Daily *ad libitum* ingestion in diet
Exposure duration	12 weeks
Average experimental exposure	0.29 mg/kg body wt-day (males); 0.33 mg/kg body wt-day (females)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	10
Intraspecies factor	10
Cumulative uncertainty factor	1,000
Oral reference exposure level	0.0003 mg/kg body wt-day (U.S. EPA RfD)

The key weaknesses in the chronic REL estimates are the lack of adequate human health data and the lack of comprehensive lifetime exposure studies in experimental animals.

While lindane did not effect reproduction and development in experimental animals at doses that produced liver and kidney effects, there is evidence that lindane may cause degeneration of reproductive organs at near subchronic dose levels. Lindane's effects on reproductive organs and the immune system should be investigated in follow-up studies.

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CHRONIC TOXICITY SUMMARY

HEXACHLOROCYCLOPENTADIENE

(1,2,3,4,5,5-Hexachloro-1,3-cyclopentadiene; HCCP; perchlorocyclopentadiene; hexachloro-1,3-cyclopentadiene)

CAS Registry Number: 77-47-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.7 μg/m^3**
Critical effect(s)	Bronchiolar epithelial erosion, focal areas of hyperplastic cuboidal and columnar epithelium, focal subepithelial fibroblastic proliferation, inflammatory cell proliferation, and/or inflammatory exudate in the lumen, lesions of olfactory epithelium, and increased mortality in rats
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB 1995)

Molecular formula	C_5Cl_6
Molecular weight	272.77 g/mol
Description	Dense, oily pale-yellow to yellow-green liquid with a unique, pungent odor.
Vapor pressure	0.080 mm Hg at 25°C
Solubility	Slightly soluble in water (2 ppm at 25°C). Soluble in acetone, carbon tetrachloride, methanol and hexane.
Conversion factor	11.16 μg/m^3 per ppb at 25°C

III. Major Uses and Sources (HSDB, 1995)

Hexachlorocyclopentadiene (HCCP) is used predominantly as an intermediate for many insecticides, polyester resins, dyes, pharmaceuticals and flame retardants. Presently, most of the chlorinated insecticides produced from HCCP have been banned, suspended or severely restricted. HCCP has no end uses of its own. Potential sources of release of HCCP include emissions and effluent discharges from facilities which manufacture or use the compound as an intermediate and from application and disposal of contaminated pesticides. Emissions of HCCP can occur from combustion of certain chlorinated wastes. HCCP is highly reactive and is not a persistent environmental contaminant. HCCP is expected to exist primarily in the vapor phase in

the atmosphere but undergoes photodegradation ($t_{1/2}$ in the atmosphere is estimated at 29 days). Potential human exposure can occur through ingestion of contaminated drinking water or contaminated fish. Exposure through the inhalation route can occur at hazardous waste disposal sites that contain this compound. Occupational exposure at facilities that manufacture or use HCCP can occur by the inhalation and dermal routes.

IV. Effects of Human Exposure

No long-term toxic exposure episodes in humans are known. However, cases of acute and short-term exposure to HCCP have occurred in humans. Unauthorized discharge of HCCP into a municipal sewer line resulted in acute exposure of sewage treatment plant workers (Morse *et al.*, 1979; Kominsky *et al.*, 1980; Meyer, 1983). Of 145 workers that were evaluated, 59% had eye irritation, 45% had headaches, and 27% had throat irritation. Other complaints were skin irritation, cough, nausea and abdominal cramps. Symptoms persisted at least 6 weeks in 5-18% of the workers. Medical examination of 41 workers three days after the plant was closed showed proteinuria and elevation of serum lactic dehydrogenase. Normal levels of these parameters were present 3 weeks later. However, a screen of urine from exposed workers 2-3 months later revealed the presence of HCCP. Health evaluations of 97 workers involved in decontamination procedures showed that they experienced similar symptoms (skin, eye and throat irritation, headaches, etc.). Nineteen percent had at least one abnormal liver function test on serial blood testing. Air concentrations of HCCP at primary treatment areas 4 days following the exposures ranged from 270 to 970 ppb.

In an HCCP epidemiological study, biochemical alterations in renal and hepatic functions were studied in 73 male operators employed at an organochlorine plant for an average of 8.2 years (range 0.5-23 years) (Boogaard *et al.*, 1993). Mean airborne concentrations of HCCP were sometimes high and occasionally exceeded the maximum allowable concentration (MAC) of 0.11 mg/m^3. The men were also exposed to several other chemicals, but only allyl chloride concentrations occasionally exceeded the MAC. The control group consisted of 35 men who were not exposed to the chemicals. No clinical effects could be related to HCCP exposure.

V. Effects of Animal Exposure

Rats exposed to ^{14}C-HCCP vapors retained, or absorbed, 83.9% of the inhaled compound (Lawrence and Dorough, 1981). Less than 1% of the radiolabel was expired as the parent compound and no $^{14}CO_2$ was detected. Thirty-three percent of the radiolabel was excreted in the urine while 23.1% was eliminated in feces. After 72 hours, 12.9% of the administered label was still present in the body. Orally administered ^{14}C-HCCP was primarily excreted in the feces (68.2% of the dose). Urinary excretion totaled 24.4% and only trace amounts of radioactivity remained in tissues after 72 hours (0.2% of the dose). Inhalation of ^{14}C-HCCP resulted in highest residue concentrations in trachea and lungs while oral administration of ^{14}C-HCCP resulted in highest residue concentrations in the kidney. The fat was not a site of residue accumulation for either route of exposure. These results indicate that the route of exposure is an

important determinant of the elimination and retention patterns. Biliary excretion of only 16% ^{14}C-HCCP, with 66% still voided in the feces, in bile duct cannulated rats suggested that the majority of orally consumed HCCP was not absorbed (Dorough and Ranieri, 1984). However, ^{14}C-HCCP eliminated in the feces was extensively metabolized. With one exception, the fate of HCCP was quite similar in rats and mice, both male and female. In rats, the kidney contained the highest levels of residues, whereas in mice, the liver contained the highest levels of residues. Residues in fat decline slowly, relative to other organs, following cessation of HCCP in diet. This study, as well as others (El Dareer *et al.*, 1983; Lawrence and Dorough, 1982), indicate that the lower toxicity of oral doses of HCCP (as compared to the inhalation route) may be related to its reaction with intestinal contents and its lack of absorption into tissues in an intact, reactive form. Injected HCCP decays from the blood biexponentially with a terminal phase half-life of 60 min (Mehendale, 1977). Subcellularly, HCCP is predominantly associated with liver and kidney cytosol fractions.

In a subchronic inhalation study, 40 Sprague-Dawley rats/group/sex and 6 cynomolgus monkeys/group/sex were exposed to 0, 0.01, 0.05 or 0.20 ppm HCCP 6 hr/day, 5 days/week for 90 days (Rand *et al.*, 1982). No treatment-related effects at any dose level were observed in monkeys. Male rats exposed to 0.05 and 0.20 ppm HCCP had dark red eyes until after the 20th exposure. Opthalmoscopic examination revealed no lesions in the eyes. Body weight gain and food consumption of rats were unaffected by HCCP exposure. Very slight, but significant, changes occurred with a few hematology indices (increased Hb, RBC count and MCHC; decreased MCV) in 0.01 ppm males, 0.05 ppm females, and 0.20 ppm males and females that may be indicative of impaired respiratory function. However, examination of the lungs found no treatment-related effects. All other hematology, clinical chemistry and urinalysis data were considered normal. Mean liver weights were reduced 3-15% among all treatment groups. Mean kidney weights were reduced 10-11% in all treated males. Upon pathologic and histopathologic examination, no abnormalities were found in these organs or any others. A preceding range-finding study (10 rats/group/sex) using the same strain of rats under similar exposure conditions observed high mortality and morbidity in male rats by 5-7 exposures at 0.5 ppm HCCP. Only a few females were affected at this dose level. Clinical signs included labored respiration, dark red eyes and paleness of extremities. A marked loss in body weight was observed in both males and females. The predominant gross pathologic finding was pale areas of consolidation in the lung. Histopathologic examination revealed lesions in the olfactory and bronchiolar epithelium and inflammatory exudate in the lumens of the respiratory tract. Fourteen-day exposure at two lower doses in the range-finding study, 0.11 and 0.022 ppm, led to no treatment-related effects. The results suggest that HCCP toxicity exhibits a steep dose-response effect and the toxic responses seen are due to direct action of HCCP on the surfaces lining the respiratory tract.

In a similar inhalation study, 27 Wistar rats/group/sex were exposed to 0, 0.06, 0.14 or 0.56 ppm HCCP 6 hr/day, 5 days/week for 30 weeks (Shell Oil Co., 1981). Nine rats/group/sex were allowed to recover for 14 weeks following cessation of exposure. Four males and two females in the high dose group died during the study. Pathology revealed the probable cause as bronchopneumonia. Clinical signs of toxicity were confined to high dose animals and consisted of occasional sneezing and lethargy during exposure periods. Body weights of the males were reduced 6-7% during most of the study. Exposed females exhibited sporadic instances of

increased weight gain. Food intake in both sexes occasionally differed from controls but could not be correlated with body weight changes. Males in the high and medium exposure groups had increased erythrocyte counts, hemoglobin concentration, hematocrits and mean absolute neutrophils. Both males (high and medium dose) and females (high dose) exhibited decreased mean absolute lymphocytes. High dose males had decreased heart weights while the two highest exposure groups had decreased spleen weights. However, these and other minor organ weight differences were considered to have no biological significance. Organ pathology was primarily found in the lungs. Gross pathology revealed pale lungs in some high dose animals while microscopic examination found degeneration of bronchiolar epithelium in all rats at this dose level. Occasional lesions in other airway epithelium and in the larynx were seen in the high dose animals. Very mild degenerative changes were occasionally seen in the liver and kidneys of high dose rats. Rats allowed to recover for 14 weeks had only occasional bronchiolar mucus deposition, perivascular fibrosis and mineralization. Otherwise, the lung lesions had resolved.

In an early series of subchronic and chronic HCCP toxicity studies, guinea pigs (two total) survived 6 weeks inhalation exposure (7 hr/day, 5 days/week) to 0.34 ppm HCCP (Treon *et al.*, 1955). However, all rats and mice (4 each) died at about this same exposure concentration by the end of the first week of exposure. Four of six rabbits died at this concentration by the fifth week of exposure. Normal growth and no mortality were seen in rats, rabbits and guinea pigs exposed to 0.15 ppm HCCP for 216 days. However, 4 of 5 mice died during this period. Clinical signs at the high dose included eyelid irritation and increased respiratory rate. Mild respiratory changes were noted in mice at the low dose; no other clinical signs were seen. Degenerative changes in livers and kidneys of all species were observed at the low dose. Irritative and inflammatory lesions in the lungs were seen in all species except rabbits at the low dose level. Contaminants may have produced some of the toxic effects; the HCCP solution used in the study was only 90% pure.

In a developmental/teratogenic toxicity study, up to 33 pregnant CF-1 mice and up to 24 pregnant New Zealand white rabbits were given 0, 5, 25, or 75 mg HCCP/kg body wt-day by gavage from days 6-15 (rats) or days 6-18 (rabbits) of gestation (Murray *et al.*, 1980). No teratogenic effect was observed in either species. Maternal and embryotoxic effects were not noted in mice, but mortality, diarrhea and significant weight loss were observed in rabbits at the high dose.

HCCP similarly showed no developmental or teratogenic potential in rats (IRDC, 1978). Pregnant Charles River CD rats were administered HCCP in corn oil by gavage at dose levels of 3,10 and 30 mg/kg body wt-day on days 6-15 of gestation. Maternal, embryotoxic, developmental, and teratogenic effects was not observed.

A 13-week subchronic study was conducted by administering HCCP in corn oil by gavage to 10 F344 rats/group/sex and 10 $B6C3F_1$ mice/group/sex at doses of 0, 10, 19, 38, 75, or 150 mg/kg body wt-day and 0, 19, 38, 75, 150, 300 mg/kg body wt-day, respectively, for 5 days/week (Abdo *et al.*, 1984). Treatment-related mortality occurred in rats at the two highest doses and in mice at the highest dose. A 10% or greater reduction in body weight compared to controls occurred in male rats and female mice at 38 mg/kg bw-day and above, and in female rats and male mice at 75 mg/kg bw-day and above. Female rats had significantly increased liver:brain weight and

kidney:brain weight at the three highest and two highest dose levels, respectively. In female mice, liver:brain weight and kidney:brain weight were significantly higher than controls at all dose levels. Lung:brain weight was also significantly increased at the highest dose. In rats and mice, gross and histopathological lesions were observed in the forestomach and the kidney. Characteristics of forestomach lesions included hyperplasia, acanthosis, and hyperkeratosis of the epithelial surface, a varying degree of inflammation, and ulceration and erosion of the mucosa. These lesions occurred in male rats at 38 mg/kg body wt-day and up, and in female rats at 19 mg/kg body wt-day and up. The severity of the injury was dose-dependent. The kidney lesions in rats were mainly limited to terminal portions of the proximal convoluted tubules in the inner cortex and were distinct from the underlying chronic rat nephropathy seen in all rat groups. Affected tubules were mildly dilated and tubular epithelial changes consisted of cytomegaly, karyomegaly and anisokaryosis with nuclear and cytoplasmic vacuolization. Other lesions included acute vascular congestion and formation of proteinaceous casts in the lower nephron. Kidney injury occurred in nearly all rats at the three highest doses of HCCP. In mice, the forestomach and kidney lesions were similar to those seen in rats. Forestomach lesions were observed at 38 mg/kg body wt-day and up in both male and female mice while the kidney lesions were observed only in the three highest doses in female mice. In addition, 7/10 male mice at the highest dose had acute tubular necrosis which may have led to their early deaths.

In a developmental/teratogenic screening study of numerous chemicals, postnatal growth and viability of prenatally exposed offspring were measured to assess developmental toxicity of HCCP (Chernoff and Kavlock, 1982). Twenty-five mice were given 45 mg/kg body wt-day by gavage on days 8-12 of gestation. Maternal toxicity was evident (3 mice died) but HCCP had no effect on litter size or pup viability up to postnatal day 3. An extension of this screening study followed postnatal viability, growth, morphology, and reproductive function of offspring exposed to HCCP prenatally (Gray and Kavlock, 1984). Under the same dosing regimen of Chernoff and Kavlock (1982), HCCP produced no long-term adverse effects in offspring up to 2 years of age.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Rand *et al.*, 1982
Study population	40 Sprague-Dawley rats/group/sex, 320 total
Exposure method	Discontinuous whole body inhalation exposure (0, 0.01, 0.05, 0.20 or 0.50 ppm)
Critical effects	Bronchiolar epithelial erosion, focal areas of hyperplastic cuboidal and columnar epithelium, focal subepithelial fibroblastic proliferation, inflammatory cell proliferation, and/or inflammatory exudate in the lumen and lesions of olfactory epithelium, and increased mortality
LOAEL	0.50 ppm
NOAEL	0.20 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	90 days
Average experimental exposure	0.036 ppm for NOAEL group (0.20 x 6/24 x 5/7)
Human equivalent concentration	0.0060 ppm (gas with extrathoracic and tracheobronchial respiratory effects, RGDR(ET) = 0.17, BW = 235 g, MV = 0.17 L/min, SA(ET) = 15 cm^2)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.00006 ppm (0.06 ppb, 0.0007 mg/m^3; 0.7 $\mu g/m^3$)

HCCP is a chemically reactive compound, producing lesions primarily at the sites of initial contact. The respiratory system is the primary target organ following inhalation exposure, and the stomach and kidney are primary targets following oral gavage. Exposure to HCCP by the general public will most likely occur through the ingestion of contaminated drinking water or ingestion of contaminated fish (HSDB, 1995). However, several researchers have shown that the inhalation route of exposure is significantly more toxic than the oral route (Treon *et al.*, 1955; El Dareer *et al.*, 1983; Lawrence and Dorough, 1982; Dorough and Ranieri, 1984). Comparison of the NOAELs of the 2 main subchronic toxicity studies (Rand *et al.*, 1982; Abdo *et al.*, 1984) reveal that the inhalation route is roughly 10 times more toxic to rats than the oral route.

The lack of a lifetime exposure study may not be a serious liability for the HCCP database. Both the 14-week and the 30-week inhalation studies observed similar adverse effects over nearly the same dose range. Both inhalation studies also show that males are more sensitive to HCCP than females. Respiratory injury was soon evident following exposure to 0.50 or 0.56 ppm HCCP,

indicating the compound's acutely toxic nature. Slightly lower concentrations of 0.14 or 0.20 ppm produced no toxic responses, indicating the steep dose-response curve for HCCP.

A weakness of the HCCP database is the lack of human pharmacokinetic studies and chronic exposure cases. However, acute poisoning cases in humans result in respiratory irritation and possibly mild liver and kidney damage.

VII. References

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CHRONIC TOXICITY SUMMARY

HEXACHLOROETHANE

(1,1,1,2,2,2-Hexachloroethane; perchloroethane; hexachloroethylene)

CAS Registry Number: 67-72-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**80 μg/m^3**
Oral reference exposure level	**0.001 mg/kg bw-day** (U.S. EPA RfD)
Critical effect(s)	Inhalation exposures: CNS effects (tremors, ataxia, hypersalivation, head bobbing and facial fasciculations), reduced body weight, increased liver, kidney, spleen, and testes weight relative to body weight in rats. (Increased respiratory lesions were interpreted as potentiation of endemic mycoplasma infection.) Oral exposures: Atrophy and degeneration of renal tubules and hypertrophy and/or dilation of proximal convoluted tubules of Fischer 344 rats.
Hazard index target(s)	Nervous system, alimentary tract, kidney

II. Chemical Property Summary (HSDB 1995)

Molecular formula	C_2Cl_6
Molecular Weight	236.74
Description	Colorless crystals with a camphor-like odor. Readily sublimes without melting
Vapor Pressure	0.4 mm Hg at 20°C
Solubility	Practically insoluble in water (0.005 g/100 g H_2O at 22.3°C). Soluble in benzene, ether, tetrachloroethylene and oils.
Conversion factor	9.68 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

The major use for hexachloroethane (HCE) is as a smoke constituent of candles and grenades for the generation of ‘smoke’ or ‘fog’(HSDB, 1995). Industrial uses include applications as a

degassing agent in production of aluminum and magnesium metals; component of extreme pressure lubricants; ignition suppressant in combustible liquids; additive to fire-extinguishing agents; and as a plasticizer for cellulose esters. HCE has been used historically as an anthelminthic to treat fascioliasis in sheep and cattle (DHHS, 1994). HCE is not known to be naturally occurring. Formation and release of HCE to the environment could occur during combustion and incineration of chlorinated wastes. Release of HCE to air could also occur due to volatility and inefficient solvent recovery and recirculation. The most probable route of human exposure is inhalation of contaminated occupational or ambient air.

IV. Effects of Human Exposure

No literature was found regarding chronic toxicity of HCE in humans. However, monitoring of HCE blood levels in munitions workers has shown exposure to the chemical can occur despite using precautions to limit inhalation and dermal exposure (Selden *et al.*, 1993). The decline of HCE blood levels in one subject indicated that the plasma half-life of HCE to be on the order of days, but less than a week. However, detectable levels of plasma HCE were found in workers exposed to HCE five weeks earlier. A few studies of human exposure under acute conditions have been published. Workmen employed in military smoke candle production and exposed to hot HCE fumes developed eye effects including blepharospasm, photophobia, lacrimation and reddening of the conjuctivae (Scherling and Blondis, 1945). No corneal injury or permanent damage occurred.

V. Effects of Animal Exposure

The distribution of HCE in the rat was studied following exposure to high levels of HCE in the diet (Gorzinski *et al.*, 1985). The highest concentrations of HCE after administration were consistently found in the fat. Concentrations in the fat were 2.5 to 5 times those found in the kidney, and over 100 times the concentrations found in the liver and the blood. Clearance of HCE from fat, liver, kidney and blood occurred in an apparent first-order manner with a half-life of about 2.5 days. The metabolic disposition of HCE was studied in rats and mice after a 4 week oral administration (5 days/week), followed by a single dose of radiolabelled HCE (Mitoma *et al.*, 1985). Forty-eight hours after the last dose, a high percentage of labeled HCE was eliminated by expiration (65% for rats; 72% for mice). This finding suggests that HCE is well absorbed but does not readily bioaccumulate. In both rodent species, less than 3% of the radiolabeled HCE was eliminated as CO_2. HCE metabolites in urine and feces of mice were over 2 times greater than those found in urine and feces of rats. The major urinary metabolites were trichloroethanol and trichloroacetic acid. In rabbits, oral administration of radiolabeled HCE resulted in only 5% of the radiolabel appearing in urine after 3 days (Jondorf *et al.*, 1957). However, 14-24% of the radioactivity, which included the parent compound and CO_2, appeared in expired air. Like the rat, the major urinary metabolites from rabbits included trichloroethanol and trichloroacetic acid. Gargas and Anderson (1989) determined that the maximum metabolic rate (V_{max}) for HCE in rats was 1.97 mg/kg/hr. Metabolism of HCE resulted in a significant decrease in cytochrome P450 levels in mice (Bronzetti *et al.*, 1989) and covalent binding to

macromolecules in rats and mice (Lattanzi *et al.*, 1988), suggesting that hepatotoxicity may occur through formation of reactive intermediates such as epoxides and free radicals. Oral administration of 500 mg/kg-bw HCE to sheep resulted in a maximum concentration in blood 24 hours later (Fowler, 1969). HCE was well distributed but biliary concentrations markedly exceeded blood concentrations. The major metabolites in sheep were pentachloroethane and tetrachloroethylene.

In a 6-week inhalation study, dogs (4 males/group), guinea pigs (15 males/group) and rats (25 animals/group/sex) were exposed to 0, 15, 48 or 260 ppm HCE 6 hr/day, 5 days/week (Weeks *et al.*, 1979). Dogs were the most sensitive mammal tested, exhibiting CNS symptoms including tremors, ataxia, hypersalivation, head bobbing and facial fasciculations during exposure to 260 ppm HCE. Guinea pigs exhibited a reduction in body weight beginning at week 2 at the highest dose. The liver-to-body weight ratio was significantly higher than controls. Body weight gain of male rats was reduced by week 3 at the highest dose. Kidney-, spleen-, and testes-to-body weight ratios of male rats and the liver ratio in females rats were significantly larger than controls. In all three species, no adverse effects were seen regarding body weights, organ weights and gross necropsy at the two lower exposure levels. No exposure-related histopathologic findings were observed in dogs and guinea pigs. Rats showed increased upper and lower respiratory lesions but this was interpreted as potentiation of an endemic mycoplasma infection.

In a 16-week subchronic study, 10 CDF Fischer-344 rats/group/sex were fed diets resulting in 0, 1, 15 or 62 mg HCE/kg body wt-day (Gorzinsky *et al.*, 1985). Body weights and food consumption were recorded at least once weekly. Routine urinalysis and hematologic parameters were conducted at the 13th week of exposure. Clinical biochemical determinations, gross pathology and histopathology were conducted at 16 weeks. The only effects observed were confined to the kidney and liver. Relative and absolute kidney weights of males fed the highest dose were about 5 and 9% greater, respectively, than controls and the differences were statistically significant. Microscopic examination revealed tubular atrophy, degeneration, hypertrophy and/or dilation at the two highest dose levels. In female rats, very slight renal tubular atrophy and degeneration were observed only at the highest dose level. Absolute and relative liver weights of males and relative liver weights of females were significantly greater at the highest dose level, but the difference from controls in all cases was less than 10%. Slight hepatocellular hypertrophy was noted in the HCE-exposed males, but was also present in some of the controls as well.

In a bioassay for possible carcinogenic and noncarcinogenic effects of technical-grade HCE (98% pure), Osborne-Mendel rats and B6C3F1 mice were administered the chemical by gavage at either of two dosages to groups of 50 male and 50 female animals of each species (USDHEW, 1978). HCE was given 5 days/week, cyclically for 44 of 78 weeks in rats and continuously for 78 weeks in mice, followed by an observation period of 33 or 34 weeks for rats and 12 or 13 weeks for mice. The high and low time-weighted average dosages of HCE were, respectively, 423 and 212 mg/kg bw-day for male and female rats and 1179 and 590 mg/kg bw-day for male and female mice. For each species, 40 controls (20 vehicle and 20 untreated) of each sex were also used. In rats, a distinct dose-related depression of mean body weight occurred in the male rat groups. Clinical signs of toxicity included a hunched appearance, reddened, squinted or

lacrimating eyes and abdominal urine stains. Significant mortality occurred in all dosed groups. In mice, few adverse effects were observed. A hunched or thin appearance was seen with greater frequency in treated groups from week 38 until termination in week 91. Histopathology revealed toxic tubular nephropathy in all groups of treated animals. In rats, this lesion was characterized by degeneration, necrosis, and the presence of large hyperchromatic regenerative epithelial cells. In mice, the nephropathy was characterized microscopically as degeneration of the convoluted tubule epithelium at the junction of the cortex and medulla. No other nonneoplastic effects were seen in either species.

In a long-term study, 50 F344/N rats/group/sex were administered HCE in corn oil by oral gavage 5 days/week for 2 years (NTP, 1989). Males received 0, 10 or 20 mg/kg bw-day while females received 0, 80 or 160 mg/kg bw-day. In male HCE-exposed rats, mean body weights were never more than 6% lower than those of vehicle controls; survival of dosed groups was similar to that of vehicle controls; and there were no clinical signs of HCE toxicity. Body weights of high dose female rats remained about 9% lower than those of the vehicle controls after week 69. Survival among females was similar among all exposure groups but some females in the high dose group were hyperactive following dosing. Adverse effects to organ systems were confined to the kidney. In males, a dose-dependent increase in kidney mineralization and hyperplasia of the pelvic transitional epithelium was observed. Renal tubule hyperplasia was increased in the high dose group. These lesions are characteristic of male rat hyaline droplet nephropathy. Nephropathy was observed in nearly all males, but the severity was slightly increased in dosed males relative to vehicle controls. In female rats, incidences of nephropathy also increased by dose and the severity of nephropathy was significantly increased in dosed animals relative to controls. However, the overall severity of nephropathy was greater in males than in females.

No multi-generation studies have been conducted with HCE in experimental animals. However, Weeks *et al.* (1979) tested for teratogenicity in rats. Groups of 22 pregnant female rats were given daily oral dosages (0, 50, 100, 500 mg/kg body wt) or exposed via inhalation (0, 15, 48 and 260 ppm) to HCE from day 6 through day 16 of gestation. The dose and concentration of HCE that produced maternal effects, 500 mg/kg body wt orally and 260 ppm by inhalation, respectively, did not produce a teratogenic effect. Only a slight slowing of fetal development occurred at the dose and concentration that produced maternal effects.

VI. Derivation of Chronic Reference Exposure Level (REL)

Inhalation Chronic Reference Exposure Level

Study	Weeks *et al.*, 1979
Study population	Dogs (4 males/group), guinea pigs (15 males/group), rats (25/group/sex)
Exposure method	Discontinuous whole-body inhalation exposure (0, 15, 48, or 260 ppm)
Critical effects	CNS effects (tremors, ataxia, hypersalivation, head bobbing and facial fasciculations), reduced body weight, increased liver, kidney, spleen, and testes weight relative to body weight. (Increased respiratory lesions were interpreted as potentiation of endemic mycoplasma infection.)
LOAEL	260 ppm
NOAEL	48 ppm
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	6 weeks
Average experimental exposure	8.6 ppm for NOAEL group (48 x 6/24 x 5/7)
Human equivalent concentration	8.6 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	10
Intraspecies factor	10
Cumulative uncertainty factor	1000
Inhalation reference exposure level	0.009 ppm (9 ppb, 0.08 mg/m^3, 80 $\mu g/m^3$)

The strengths of the inhalation REL include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis, and the observation of a NOAEL. Major uncertainties include the lack of human data and the lack of chronic exposure inhalation studies.

Oral Chronic Reference Exposure Level

Study	Gorzinsky *et al.*, 1985
Study population	10 rats/group/sex, 80 total.
Exposure method	Ingestion, in diet *ad libitum* (resulting in 1, 15 or 62 mg HCE/kg bw-day).
Critical effects	Atrophy and degeneration of renal tubules (males and females); hypertrophy and/or

	dilation of proximal convoluted tubules (males only)
LOAEL	15 mg/kg bw-day
NOAEL	1 mg/kg bw-day
Exposure continuity	Ingestion, in diet *ad libitum* 15 hr/day
Exposure duration	16 weeks
Average experimental exposure	1 mg/kg bw-day
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	10
Intraspecies factor	10
Cumulative uncertainty factor	1000
Oral reference exposure	0.001 mg/kg bw-day (U.S. EPA RfD)

The primary reasons the oral REL for HCE is based on the study by Gorzinsky *et al.* (1985), rather than on the comprehensive lifetime NTP (1989) study, are (1) a lower NOAEL was determined for HCE in the Gorzinsky study and (2) nephropathy was noted in most NTP males, including controls, which confounds that study. The lifetime HCE exposure study observed an increase in severity of nephropathy in the high dose male rats (20 mg/kg bw-day) and an increase in the incidence and severity of nephropathy in dosed females (80 and 160 mg/kg bw-day). Therefore, the lifetime study has a NOAEL of 10 mg/kg bw-day for males while the exposure data for females did not observe a NOAEL. The Gorzinsky study observed a significant increase in nephropathy of male rats at 15 mg/kg bw-day, but not at the lowest dose of 1 mg/kg bw-day. It should be noted, however, that some of the adverse effects observed in the kidneys of male rats may be specific only to male rats (known as male rat-specific hyaline droplet syndrome) and not relevant to humans (Phillips and Cockrell, 1984; Beyer, 1992).

Weaknesses of the Gorzinsky *et al.* (1985) study include small sample sizes. Because of the rat-specific nephropathy seen with HCE exposure, as well as with many other chlorinated compounds, the database would be enhanced if a comprehensive subchronic or chronic study was performed with a non-rodent species. While a few studies have looked at teratogenicity of HCE, no multi-generation studies have been performed to investigate potential developmental or reproductive effects. Another weakness is the lack of human toxicity data for this compound.

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CHRONIC TOXICITY SUMMARY

HEXAMETHYLENE DIISOCYANATE

(HMDI; HDI; 1,6-diisocyanatohexane)

CAS Registry Number: 822-06-0

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.01 μg/m^3** (U.S. EPA RfD) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
Critical effect(s)	Degeneration of olfactory epithelium in the rat
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	$C_8H_{12}N_2O_2$
Molecular weight	168.2 g/mol
Description	Colorless to slightly yellow liquid
Vapor pressure	0.05 mm Hg @ 25°C
Solubility	Low solubility in water (reacts)
Conversion factor	6.88 μg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Hexamethylene diisocyanate (HDI) is used as a chemical crosslinking agent in the production of a large number of polymer products including polyurethane paints (for its curing/hardening properties), polyurethane foams, adhesives, elastomers, dental materials, contact lenses, biuret polyisocyanate resins, and medical adsorbants (HSDB, 1995). Most two component paints use HDI in the form of non-volatile prepolymers (Vandenplas *et al.*, 1993). Residual unreacted (monomeric) hexamethylene diisocyanate in products may be present at levels of 0.5-1.0%. Hexamethylene diisocyanate biuret trimer (HDI-BT), a polycondensation product of HDI and water, is also used in conjunction with HDI as car paint hardener. The presence of unpolymerized HDI indicates a potential source of environmental release and exposure, such as during the application of paint.

IV. Effects of Human Exposure

A respiratory sensitization effect, termed "isocyanate asthma", is associated with repeated occupational exposure to isocyanates, including HDI.

The case of a worker in a paint shop exposed to multiple solvents and paint components including several esters, xylene, toluene, and HDI has been reported (Malo *et al.*, 1983). Over the course of 5-6 years the worker reported episodes of shortness of breath, wheezing, malaise, and chills while working. Examination three weeks after the most recent exposure to HDI, lung function test results were found to be in the normal range. Five minute inhalation challenges with enamel with and without HDI (separated by a three week recovery period) resulted in a burning sensation in the chest and cough within one hour. The maximum air concentration of HDI was 0.02 ppm. Over the next six hours, the subject reported symptoms of hypersensitivity pneumonitis including productive cough, chills, headache, malaise, and increased leukocyte count, ultimately leaving the subject prostrate. Lung function tests suggested alveolar and bronchial involvement, as well as hyperinflation and airway obstructions, in the response to HDI. Bronchial hyperexcitability continued until 53 days after the initial challenge. IgG antibodies against HDI were also detected in the subject's serum. IgG antibodies against polyisocyanates have also been reported in a group of 30 car painters exposed to HDI monomer and prepolymer (Welinder *et al.*, 1988). Hypersensitivity pneumonitis and late asthmatic reactions to HDI have also been reported (Zeiss *et al.*, 1983; Innocenti *et al.*, 1986).

Occupational asthma and immunoglobulin production from exposure to isocyanates were reported (Cartier *et al.*, 1989). Of 62 exposed workers (63% to HDI, 27% to diphenylmethane diisocyanate, 10% to toluene diisocyanate), inhalation challenges were conducted for periods ranging from one breath to 120 minutes with commercial preparations of HDI. For the final 28 subjects, isocyanate levels in air were monitored and fell generally in the range of 5 and 20 ppb. No irritation symptoms (cough, rhinitis, conjunctivitis, nausea, or headache) were reported from these levels of exposure. Spirometry, body temperature, and blood cell counts Three subjects showing positive challenge test results to HDI had exposure levels quantified. One showed an immediate asthmatic reaction after one breath at 5 ppb HDI, one showed a late asthmatic reaction with 6 minutes of exposure to 13.0 ppb ± 4.0 (s.d.), and a third had a late asthmatic reaction with 5 minutes of exposure to 12.3 ppb ± 3 (s.d.). Increased levels of anti-isocyanate IgG and/or IgE were found to be associated with positive inhalation challenges.

A study of 41 Swedish car painters exposed to HDI and HDI-BT was conducted (Alexandersson *et al.*, 1987). Control groups included car platers (48) who were exposed to solvents and paint dust but not HDI, and car mechanics (70) who were reportedly unexposed. The mean time of employment was 7 years. The mean estimated level of air exposure was 115 $\mu g/m^3$ HDI-BT and approximately 1 $\mu g/m^3$ HDI. No differences in pulmonary function were found between the groups tested, however, an increase in closing volume relative to vital capacity, which tended to increase during the course of the work week, among car painters relative to controls suggested a condition known as "small airway disease". A follow-up study examined 36 car painters 6 years after the initial study (Tornling *et al.*, 1990). Revised estimated exposure level were found to be

90 $\mu g/m^3$ HDI-BT and 1.5 $\mu g/m^3$ HDI. Among cigarette smoking car painters relative smoking controls, forced vital capacity, one second forced expiratory volume, and vital capacity were reduced, an effect not seen among the non-smoking groups. This effect was found to be correlated with the frequency of high peak exposures to HDI-BT.

V. Effects of Animal Exposure

Mobay, Inc. has conducted several inhalation studies exposing rats to HDI, with the most thorough evaluation of chronic toxicity reported in 1989 (Mobay, Inc., 1989). The study was found to conform to U.S. EPA TSCA guidelines for toxicity and oncogenicity studies (Stern, 1990). Groups of 60 Fischer 344 rats/sex were exposed to 0, 0.005, 0.025, or 0.164 ppm HDI for 6 hrs/day, 5 days/week for 2 years and satellite groups of 10 rats/sex were similarly exposed for one year. Rats in the high-dose group were initially exposed to 0.126 ppm HDI for the first 127 days, but exposure levels were increased to 0.172 ppm for the remainder of the experiment due to the absence of overt signs of toxicity. The resulting time-weighted exposure level was 0.164 ppm HDI.

No significant differences were reported between HDI-exposed and control animals with respect to mortality, clinical chemistry, and urinalysis. Animals in the high dose group showed occasional elevations in reticulocyte counts, possibly indicating anemia. Other blood cell counts were generally not significantly different from controls. Eye irritation was reported during the first year of exposure in male animals receiving the high dose, but no lesions of the eye were found by ophthalmic examination.

Lung and respiratory tract effects were most clearly associated with HDI exposure. The 0.025 and 0.164 ppm HDI dose groups showed lesions of the lung characterized by increased epithelial content, interstitial pneumonia, and accumulation of histiocytes. Similarly, lesions of the nasal epithelium in the two high dose groups were described as hyperkeratosis, occasional atrophy, and focal ulceration of the olfactory epithelium. The nasal cavity lesion sites included the vestibule, prepapilla, posterior incisor, and the first palatal ridge. The incidence and severity of olfactory epithelium degeneration in the first palatal ridge were reported to be HDI concentration dependent. Other nasal lesions occurring in the low- and mid-exposure groups with increased incidence over controls included hyperplasia/metaplasia, mucus hyperplasia, and inflammation. In the satellite groups exposed to HDI for one year, similar effects to those in the two year study were observed, although the severity and the incidence were not as high.

Mobay, Inc. also conducted a 13-week study exposing Fischer 344 rats (20/sex/group) to HDI vapor at concentrations of 0, 0.01, 0.04, and 0.14 ppm for 6 hours/day, 5 days/week (Mobay, Inc., 1984). Observations made included body weight, hematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic pathology. Eye irritation was observed in all of the HDI-exposed groups. Minimal signs of degeneration of the olfactory epithelium were observed in two of the 20 male rats in the high dose group. Other observations made included inflammation and mucus hyperplasia, but these effects were not found to be dose-related in severity or incidence. All HDI-exposed groups showed squamous metaplasia of the respiratory

tract described as keratinized and disorganized, and the effect was found to be dose-dependent in incidence and severity. No control animals showed this lesion.

An additional study was conducted by Mobay, Inc., exposing Sprague-Dawley rats (10/sex/group), head only, to 0, 0.005, 0.0175, 0.15, and 0.3 ppm HDI for 5 hours/day, 5 days/week for 3 weeks (Mobay, Inc., 1984). Half of each group was sacrificed at the end of the exposure, and half was sacrificed after a two week recovery period. The same observations were performed as in the 13-week study. Eye and nose irritation were noted in groups exposed to 0.0175 ppm HDI and greater in a concentration dependent manner. Observed effects included decreased absolute kidney weight in females exposed to 0.0175 ppm HDI or greater and males in the highest dose group. Relative kidney weights were decreased in groups of males and females in the highest dose group. Absolute and relative liver weights were decreased in females in the 0.0175 and 0.3 ppm HDI dose groups. Observed histological effects on the nasal turbinates included hemorrhage (males at 0.15 and 0.3 ppm), acute inflammation (both sexes at 0.15 and 0.3 ppm), squamous metaplasia (both sexes at 0.0175, 0.15 and 0.30 ppm), and necrosis of the epithelium (males in all dose groups; females at 0.15 and 0.30 ppm). Incidence of inflammation of the trachea was increased in all exposed female groups and in the 0.15 and 0.30 ppm HDI-exposed male groups.

Respiratory effects have been observed in animals exposed to HDI by inhalation for shorter exposure periods. Decreased respiratory rates, dyspnea, and rales were observed in rats exposed to 1.17 ppm HDI for 3 hours/day for 5 days (Mobay, Inc., 1987). Animals exposed to lethal concentrations of HDI have also shown symptoms of respiratory distress, bronchopneumonia, and hemorrhage of the lung (Mobay, Inc. and Desmodor, 1970; Mobay, Inc., 1971; Mobay, Inc., 1987; Karol *et al.*, 1984).

VI. Derivation of U.S. EPA RfC

Study	Mobay, Inc., 1989
Study population	Rats (60/sex/group)
Exposure method	Discontinuous inhalation exposures (0, 5, 25, 164 ppb) over 2 years
Critical effects	Degeneration of olfactory epithelium
LOAEL	25 ppb
NOAEL	5 ppb
Exposure continuity	6 hr/day, 5 days/ week
Exposure duration	2 years
Average experimental exposure	0.89 ppb for NOAEL group
Human equivalent concentration	0.16 ppb (gas with extrathoracic respiratory effects, RGDR(ET) = 0.18)
LOAEL uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factor	3 (inadequate data)

Cumulative uncertainty factor	100
Inhalation reference exposure level	0.002 ppb (0.01 μg/m^3)

The Mobay (1989) study was chosen for the derivation of the chronic reference exposure level because it represents the most thorough reporting of toxic effects from chronic exposure to HDI and was found to conform to U.S. EPA TSCA standards for toxicity studies. Studies in humans, particularly those describing sensitization to isocyanates, do not present adequate quantitation of the exposure levels which led to the sensitization for development of a chronic reference exposure level.

Findings among the animal studies are consistent, although a single reporting group (Mobay, Inc.) is responsible for most of the useful animal toxicological data from inhalation of HDI. Specifically, consistently reported effects include irritation of the eyes and lesions of the olfactory epithelium. Lesions within the nasal turbinates and changes in the epithelium of the respiratory tract of rats exposed to HDI have shown a dose-response relationship (Mobay, Inc., 1989; Mobay, Inc., 1984).

Acute human exposure to HDI has been reported to be irritating to the eyes, consistent with that reported in animals (Mobay, Inc., 1989).

The strengths of the inhalation REL include the availability of chronic inhalation exposure data from a well-conducted study with histopathological analysis, the demonstration of a dose-response relationship, and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data and the lack of reproductive and developmental toxicity studies.

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CHRONIC TOXICITY SUMMARY

N-HEXANE

(normal hexane)

CAS Registry Number: 110-54-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.2 mg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
Critical effect(s)	Neurotoxicity; electrophysiological alterations in humans
Hazard index target(s)	Nervous system

II. Physical and Chemical Properties (HSDB, 1995)

Molecular formula	C_6H_{14}
Molecular weight	86.10
Description	Colorless liquid, gas
Specific gravity	0.660 @ 20° C
Boiling point	68.95°C
Vapor pressure	150 mm Hg @ 25° C
Solubility	insoluble in water; soluble in most organic solvents; very soluble in alcohol
Conversion factor	1 ppm = 3.52 mg/m^3 @ 25° C

III. Major Uses or Sources

n-Hexane is used in the extraction of vegetable oil from seeds such as safflower, soybean, cotton, and flax (HSDB, 1995). It is also used as a alcohol denaturant and as a paint diluent. The textile, furniture and leather industries use n-hexane as a cleaning agent. Many petroleum and gasoline products contain n-hexane.

IV. Effects of Human Exposure

An epidemiologic study was performed on workers employed in a factory producing tungsten carbide alloys exposed for and average of 6.2 years to solvent vapors consisting of an 8-hour time weighted average of 58 ppm (±41 ppm) n-hexane and 39 ppm (±30 ppm) acetone (Sanagi *et al.*, 1980). Neurological examinations performed on both control and exposed workers examined

cranial nerves, motor and sensory nerves, reflexes, coordination and gait. Neurophysiological and nerve stimulation studies were also performed. While no overt neurological abnormalities were noted, the mean motor nerve conduction velocity and residual latency of the exposed group were significantly decreased as compared to unexposed workers. The effects observed are consistent with other reports of n-hexane-induced peripheral neuropathy. The study reports a LOAEL of 58 ppm n-hexane.

Polyneuropathy with subsequent development of muscular atrophy and paresthesia in the distal extremities was observed in workers exposed to between 500 and 1000 ppm n-hexane in a pharmaceutical plant (Yamada, 1967).

V. Effects of Animal Exposure

Mice were exposed to 500, 1000, 4000, or 10000 ppm n-hexane 6 hours per day, 5 days per week for 13 weeks or to 1000 ppm n-hexane for 22 hours per day, 5 days per week for 13 weeks (Dunnick *et al.*, 1989). Mild inflammatory, erosive and regenerative lesions in the olfactory and respiratory epithelium were observed in the nasal cavity of mice exposed to 1000 ppm n-hexane and higher. "Minimal lesions" were noted in those mice exposed to 500 or 1000 ppm n-hexane. Paranodal axonal swelling in the tibial nerve were observed in 6/8 mice exposed to 1000 ppm for 22 hours per day and in 6/8 mice exposed to 10000 ppm for 6 hours per day. No such swelling was noted in neurohistological examination of the control animals; neurohistological examination was not performed in those animals exposed to 500 and 1000 ppm for 6 hours per day. A NOAEL for histological lesions of the nasal turbinates of 500 ppm n-hexane was identified. Because neurohistological examinations were not performed in animals exposed to 500 or 1000 ppm (the NOAEL and LOAEL, respectively), the interpretation of the results from this study are seriously limited.

Dose-related signs of neurotoxicity, as measured by electromyography was observed in male mice continuously exposed to 250, 500, 1000, or 2000 ppm commercial grade hexane (65-70% n-hexane) 6 days per week for 1 year (Miyagaki, 1967). Abnormal posture and muscle atrophy were also observed in a dose-related manner in mice exposed to 250 ppm n-hexane or higher. No adverse effects were detected in the 100 ppm exposure group.

A dose-dependent decrease in motor nerve conduction velocity and body weight gain was observed in rats exposed to 500, 1200, or 3000 ppm n-hexane for 12 hours per day, 7 days per week for 16 weeks (Huang *et al.*, 1989). The neurotoxicity was significant in the two highest exposure groups; peripheral nerve degeneration, characterized by paranodal swellings and demyelination and remyelination in the myelinated nerve fibers was observed and was more advanced in the highest exposure group.

Available studies indicate that the neurotoxicity of n-hexane is potentiated by concurrent exposure to methyl ethyl ketone (Altenkirch *et al.*, 1982).

Pregnant rats were exposed to 200, 1000, or 5000 ppm n-hexane 20 hours per day on days 9-19 of gestation (Mast *et al.*, 1987). A statistically significant decrease in fetal body weight compared to controls was observed in male offspring following maternal exposure to 1000 and 5000 ppm n-hexane. Maternal toxicity, indicated by decreased body weight gain, was observed in all exposure groups.

Rabbits exposed to 3000 ppm n-hexane for 8 hours per day, 5 days per week for 24 weeks developed exposure-related lesions of the respiratory tract with the terminal bronchioles exhibiting the most characteristic damage (Lungarella *et al.*, 1984). Clinical signs of ocular and upper respiratory tract irritation and respiratory difficulties (such as gasping, lung rales, mouth breathing) were observed throughout the study in exposed rabbits.

VI. Derivation of U.S. EPA RfC

Study	Sanagi *et al.*, 1980; U.S. EPA, 1995
Study population	Tungsten carbide alloy production workers
Exposure method	Discontinuous inhalation (occupational)
Critical effects	Decreased motor nerve conduction velocity; increased residual latency
LOAEL	58 ppm (204 mg/m^3)
NOAEL	Not observed
Exposure continuity	8 hours/day (10 m^3/day occupational inhalation rate), 5 days/week
Exposure duration	6.2 years
Average occupational exposure	10.4 ppm (36 mg/m^3) for LOAEL group
Human equivalent concentration	10.4 ppm (36 mg/m^3)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
LOAEL uncertainty factor	10
Modifying factor	3 (database deficiencies for reproductive effects)
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.2 mg/m^3 (200 $\mu g/m^3$; 60 ppb; 0.06 ppm)

A statistically significant decrease in mean motor nerve conduction velocity (MMCV) and a statistically significant increase in residual latency (RL) was observed exposed workers compared to unexposed workers (Sanagi *et al.*, 1980). The LOAEL for electrophysiological alterations in exposed workers was 58 ppm n-hexane. No NOAEL was apparent from this study.

The major strength of the RfC is the use of human health effects data. The major limitations are the lack of dose-response information or the observation of a NOAEL, the uncertainties associated with the pattern and magnitude of exposures, and the limited range of possible health effects that were addressed.

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CHRONIC TOXICITY SUMMARY

HYDRAZINE

(diamine; diamide; nitrogen hydride; levoxine)

CAS Registry Number: 302-01-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.2 µg/m^3**
Critical effect(s)	Amyloidosis of the liver and thyroid in hamsters
Hazard index target(s)	Alimentary system; endocrine

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	N_2H_4
Molecular weight	32.05 g/mol
Description	Colorless, oily liquid; white crystals
Vapor pressure	14.4 mm Hg @ 25°C
Solubility	Miscible with water, methyl-, ethyl-, isobutyl alcohols; slightly miscible with hydrocarbons; insoluble in chloroform, ether
Conversion factor	1.31 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Hydrazine is a highly reactive base and reducing agent. Its primary uses are as a high-energy rocket propellant, as a reactant in military fuel cells, in nickel plating, in the polymerization of urethane, for removal of halogens from wastewater, as an oxygen scavenger in boiler feedwater to inhibit corrosion, and in photographic development (Von Burg and Stout, 1991). Hydrazine was historically used experimentally as a therapeutic agent in the treatment of tuberculosis, sickle cell anemia, and non-specific chronic illnesses (Von Burg and Stout, 1991; Gold, 1987).

IV. Effects of Human Exposure

A single case report documents toxic effects in a human from chronic inhalation/dermal exposure to hydrazine. One person was occupationally exposed to hydrazine at unknown levels on a weekly basis for a period of 6 months (Sotaniemi *et al.*, 1971). The worker showed symptoms of

conjunctivitis, tremors, and lethargy. Vomiting, fever, and diarrhea developed on the last day of exposure which progressed to abdominal pain and incoherence. The individual died three weeks after the last exposure. Evidence of tracheitis, bronchitis, heart muscle degeneration, and liver and kidney damage were found at autopsy.

Dermal sensitization has also been reported from repeated contact with hydrazine (Van Ketal, 1964; Von Keilig and Speer U, 1983; Wrangsjo and Martensson, 1986).

V. Effects of Animal Exposure

An inhalation study of the toxicity and carcinogenicity of hydrazine was conducted in cats, mice, hamsters, and dogs (Vernot *et al.*, 1985). Various animal groups were exposed for one year to concentrations of 0.05, 0.25, 1.0, and 5.0 ppm anhydrous hydrazine base for 6 hours/day, 5 days/weeks, excepting weekends and holidays. Exposed and controls groups were made up of the following animals: 100 Fischer 344 rats/sex at 0.05, 0.25, 1.0 and 5.0 ppm hydrazine plus 150 rats/sex as controls; 400 female C57BL/6 mice at 0.05, 0.25, and 1.0 ppm hydrazine plus 800 female mice as controls; 200 male Golden Syrian hamsters at 0.25, 1.0, and 5.0 ppm hydrazine plus 200 male hamsters as controls; 4 beagle dogs/sex at 0.25 and 1.0 ppm hydrazine plus 4 dogs/sex as controls. Animals were observed post-exposure for the following periods: 18 months for rats, 15 months for mice, 12 months for hamsters, and 38 months for dogs. Animals were observed hourly during the exposure period and daily in the post-exposure period.

No non-cancer toxic effects were observed in mice or dogs, with the exception of a single dog exposed to 1.0 ppm hydrazine which showed cyclic elevations in serum glutamic-pyruvic transaminase levels and, upon necropsy at 36 months post-exposure, showed liver effects described as “clusters of swollen hepatocytes that had highly vacuolated cytoplasm”. Of the other species examined, hamsters showed toxicity at the lowest dose levels, particularly amyloidosis in various organs including liver, spleen, kidney, thyroid, and adrenal glands. An increased incidence of amyloidosis was seen at the lowest exposure level (0.25 ppm hydrazine) in the liver and thyroid (67/160 exposed vs. 42/180 control for the liver and 20/117 exposed vs. 15/179 control in the thyroid; $p \leq 0.01$ by Fisher’s exact test). This effect was found to be dose related. The incidence of hemosiderosis of the liver was also significantly increased in all exposed groups. Significantly increased incidences of toxic effects observed in the 1.0 and 5.0 ppm hydrazine groups include amyloidosis of the spleen, kidney glomerulus, and adrenals glands, and lymphadenitis of the lymph nodes. Significantly increased toxic effects observed only in the highest dose group include amyloidosis of the kidney interstitium and thyroid, and senile atrophy of the testis. The authors note these effects appear to reflect accelerated changes commonly associated with aging in hamsters.

In the hydrazine exposed rats, effects were observed in the respiratory tract of exposed animals. Specifically, squamous metaplasia of the larynx, trachea, and nasal epithelium (males only) was observed in the highest dose group (5.0 ppm hydrazine). Inflammation was also observed in the larynx and trachea of rats exposed to 5.0 ppm hydrazine. Increased incidence of focal cellular change of the liver was observed in female mice at 1.0 and 5.0 ppm hydrazine. Other effects

with incidence found to be increased only in the high dose group include hyperplastic lymph nodes in females, endometriosis, and inflammation of the uterine tube.

The toxic effects from inhalation of hydrazine over a six month period from both intermittent and continuous exposure scenarios were examined (Haun and Kinkead, 1973). Groups of 8 male beagle dogs, 4 female rhesus monkeys, 50 male Sprague-Dawley rats, and 40 female ICR rats per dose group were continuously exposed to 0.2 or 1.0 ppm hydrazine or intermittently (6 hours/day, 5 days/week) to 1.0 or 5.0 ppm hydrazine. A control group consisted of equal numbers of animals. The experimental design was such that each intermittent exposure group had a time-weighted-average matching continuous exposure group. Dose-related body weight reductions were observed in all treated groups as well as evidence of hepatic degeneration, fatty deposition in the liver, central nervous system depression and lethargy, eye irritation, and anemia.

Toxic effects from the exposure of rats, mice, and dogs to airborne hydrazine at levels of 0, 4.6, or 14 ppm intermittently for 6 months were reported (Comstock *et al.*, 1954). Observed adverse effects included anorexia, irregular breathing, vomiting, fatigue, and emphysema in dogs; pulmonary congestion and emphysema in rats and mice; and lung and liver damage in rats.

Lymphoid bronchial hyperplasia was observed in guinea pigs exposed to 2-6 ppm hydrazine for 5 days/week for 19-47 days (Weatherby and Yard, 1955).

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Vernot *et al.*, 1985
Study population	Hamster
Exposure method	Inhalation
Critical effects	Amyloidosis and hemosiderosis of the liver; thyroid amyloidosis
LOAEL	0.25 ppm
NOAEL	Not observed
Exposure continuity	6 hour/day, 5 days/week
Exposure duration	6 months
Average experimental exposure	0.045 ppm for LOAEL group
Human equivalent concentration	0.045 ppm for LOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Subchronic uncertainty factor	1
LOAEL uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.0001 ppm (0.1 ppb, 0.0002 mg/m^3, 0.2 $\mu g/m^3$)

Vernot *et al.*(1985) present a thorough examination of chronic health effects from inhalation exposure to hydrazine. This study was chosen for the development of the chronic reference exposure level because (1) it was conducted with an adequate number of animals, (2) the critical/sensitive adverse effect (degenerative change in the liver in hamsters) showed a dose-response relationship, (3) the findings of this study support data found in studies by other groups.

This study shows a dose-related increase in the incidence of amyloidosis and hemosiderosis in hamsters intermittently exposed by inhalation to levels of hydrazine greater than 0.25 ppm. Other effects noted at 0.25 ppm included weight depression during exposure, mineralization of the kidney, and amyloidosis of the thyroid. Haun and Kinkead (1973) have also noted lesions of the liver in dogs, monkeys, and mice exposed to hydrazine for 6 months by inhalation. Comstock *et al.* (1954) observed liver damage in groups of rats exposed to hydrazine vapors. The single case report of hydrazine inhalation toxicity in humans showed necrosis and degeneration of the liver (Sotaniemi *et al.*, 1971).

The strengths of the inhalation REL include the availability of chronic inhalation exposure data from a well-conducted study with histopathological analysis. Major areas of uncertainty are the lack of adequate human exposure data, the lack of reproductive and developmental toxicity studies, and the lack of observation of a NOAEL.

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CHRONIC TOXICITY SUMMARY

HYDROGEN CHLORIDE

(Hydrochloric acid; anhydrous hydrogen chloride; muriatic acid)

CAS Registry Number: 7647-01-0

I. Chronic Reference Exposure Level

Inhalation reference exposure level	**7 µg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Hyperplasia of nasal mucosa, larynx, and trachea in rats
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1995)

Description	Colorless gas
Molecular formula	HCl
Molecular weight	36.46
Specific gravity	1.05 @ 15° C
Boiling point	-84.9° C (HCl gas)
Melting point	-114.8° C (HCl gas)
Vapor pressure	760 mm Hg @ -84.3° C
Solubility	Soluble in water, alcohol, benzene, ether; insoluble in hydrocarbons
Conversion factor	1 ppm = 1.49 mg/m^3 at 25°C

III. Major Uses or Sources

Hydrogen chloride (HCl) is used in the manufacture of vinyl chloride, fertilizers, dyes, artificial silk, and pigments for paints. It is also used in electroplating, soap refining, and leather tanning. Other consumers of HCl include the photographic, textile and rubber industries (HSDB, 1994).

Hydrogen chloride is produced in large quantities during combustion of most materials and especially materials with a high chlorine content. Thus, HCl is a major product formed during the thermal decomposition of polyvinyl chloride, a commonly used plastic polymer (Burleigh-Flayer *et al.*, 1985). It is also released in large quantities during the test firing of some rocket and missile engines (Wohlslagel *et al.*, 1976).

IV. Effects of Human Exposure

Few reports are available on the effects of chronic HCl exposure on humans. Bleeding of the nose and gums and ulceration of the mucous membranes was observed following repeated occupational exposure to HCl mist at high but unquantified concentrations (Stokinger, 1981). In another report, workers exposed to various mineral acids, including HCl exhibited etching and erosion of the front teeth (Ten Bruggen Cate, 1968).

V. Effects of Animal Exposure

Male rats were exposed to 10 ppm HCl 6 hours per day, 5 days per week for their lifetime (Sellakumar *et al.*, 1985). No differences in body weights or survival were observed between exposed and control animals. Increased incidences of hyperplasia of the nasal mucosa, larynx, and trachea were observed in exposed rats compared to controls.

A 90-day inhalation study in mice and rats exposed to 10, 20, or 50 ppm HCl 6 hours per day, 5 days per week for 90 days (Toxigenics, 1984). Concentration- and time-related lesions were noted in the anterior portion of the nasal cavity of exposed rats. Cheilitis, eosinophilic globules in the nasal epithelium and accumulation of macrophages in the peripheral tissues were observed in mice of all exposed groups. This study identified a LOAEL in both mice and rats of 10 ppm. The U.S. EPA considered this study supportive of the portal-of-entry effects observed at 10 ppm in the lifetime rat study (Sellakumar *et al.*, 1985).

Female rats exposed to 302 ppm HCl for 1 hour either 12 days prior to mating or on day 9 of gestation exhibited severe dyspnea and cyanosis; the exposure was lethal to one-third of the exposed animals (Pavlova, 1976). Fetal mortality was significantly higher in rats exposed during pregnancy. Organ functional abnormalities observed in offspring exposed at 2-3 months of age were reported to be similar to those observed in the exposed dams.

VI. Derivation of U.S. EPA Reference Concentration

Study	Sellakumar *et al.*, 1985
Study population	Rats (100 males)
Exposure method	Discontinuous whole-body inhalation (0 or 10 ppm)
Critical effects	Hyperplasia of the nasal mucosa, larynx and trachea
LOAEL	10 ppm
NOAEL	Not identified
Exposure continuity	6 hours per day, 5 days per week
Average experimental exposure	1.8 ppm for LOAEL group
Human equivalent concentration	4.6 ppm (gas with extrathoracic and

	tracheobronchial respiratory effects, RGDR = 2.6, based on MV = 0.5 m^3/day, SA(ET+TB) = 49.2 cm^2)
Exposure duration	Lifetime
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	1,000
Reference Concentration (RfC)	0.005 ppm (5 ppb; 0.007 mg/m^3; 7 µg/m^3)

U.S. EPA evaluated this RfC as a having a low level of confidence because of (1) the use of only one dose; (2) limited toxicity evaluation; (3) the lack of reproductive toxicity data; and (4) the lack of chronic exposure studies (U.S. EPA, 1994).

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CHRONIC TOXICITY SUMMARY

HYDROGEN CYANIDE

(*Formonitrile; hydrocyanic acid; prussic acid)*

CAS Registry Number: 74-90-8

I. Chronic Toxicity Summary

Inhalation reference exposure level	**3 µg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	CNS effects, thyroid enlargement, and hematological disorders
Hazard index target(s)	Nervous system; endocrine system; cardiovascular system

II. Physical and Chemical Properties (HSDB, 1994)

Description	Colorless liquid/gas
Molecular formula	HCN
Molecular weight	27.03
Boiling point	25.6 ^{o}C
Melting point	-13.4 ^{o}C
Vapor pressure	630 mm Hg @ 20^{o}C
Solubility	Miscible in water, alcohol; slightly soluble in ether
Conversion factor	1 ppm = 1.10 mg/m^3 @ 25 ^{o}C

III. Major Uses or Sources

Hydrogen cyanide is used in a variety of syntheses including the production of adiponitrile (for nylon), methyl methacrylate, sodium cyanide, cyanuric chloride, chelating agents, pharmaceuticals, and other specialty chemicals. Manufacturing activities releasing hydrogen cyanide include electroplating, metal mining, metallurgy and metal cleaning processes. Additionally, hydrogen cyanide has some insecticide and fungicide applications (ATSDR, 1993). Fires involving some nitrogen-containing polymers, often found in fibers used in fabrics, upholstery covers, and padding, also produce hydrogen cyanide (Tsuchiya and Sumi, 1977).

Another common source of hydrogen cyanide is cigarette smoke. Levels in inhaled mainstream cigarette smoke range from 10 to 400 µg per cigarette (U.S. brands), 0.6% to 27% (w/w) of these mainstream levels are found in secondary or sidestream smoke (Fiskel *et al.*, 1981).

IV. Effects of Human Exposure

Occupational epidemiological studies investigating hydrogen cyanide exposure are complicated by the mixed chemical environments created by synthetic and metallurgic processes. However, several reports indicate that chronic low exposure to hydrogen cyanide can cause neurological, respiratory, cardiovascular, and thyroid effects (Blanc *et al.*, 1985; Chandra *et al.*, 1980; El Ghawabi *et al.*, 1975). Although these studies have limitations, especially with incomplete exposure data, they also indicate that long-term exposure to inhaled cyanide produces CNS and thyroid effects.

El Ghawabi *et al.* (1975) studied 36 male electroplating workers in three Egyptian factories exposed to plating bath containing 3% copper cyanide, 3% sodium cyanide, and 1% sodium carbonate. Breathing zone cyanide concentrations ranged from 4.2 to 12.4 ppm (4.6 to 13.7 mg/m^3), with a mean 6.4 to 10.4 ppm (7.1 to 11.5 mg/m^3), in the three factories at the time of this cross-sectional study. The men were exposed for a duration of 5 to 10 years, with one man having 15 years exposure. Twenty non-exposed male volunteers were used as controls. None of the subjects, controls or workers, currently smoked cigarettes. Complete medical histories were taken, and medical exams were performed. Urinary levels of thiocyanate (a metabolite of cyanide) were utilized as a biological index of exposure. Thyroid function was measured as the uptake of radiolabeled iodine, since thiocyanate may block the uptake of iodine by the thyroid leading to iodine-deficiency goiters (Hartung, 1983). Frequently reported symptoms in the exposed workers included headache, weakness, and altered sense of taste or smell. Lacrimation, abdominal colic, and lower stomach pain, salivation, and nervous instability occurred less frequently. Twenty of the thirty six exposed workers had thyroid enlargements, and the thyroid function test indicated significant differences in uptake between controls and exposed individuals after 4 and 24 hours. Urinary excretion of thiocyanates correlated with the breathing zone concentrations of cyanides. This study reported a LOAEL of 6.4 ppm (7.1 mg/m^3) for the CNS symptoms and thyroid effects.

Dyspnea was observed in workers chronically exposed (5 to 15 years) to 6.4 to 10.4 ppm (7.0 - 11.4 mg/m^3) of an unspecified cyanide form produced from sodium cyanide and copper cyanide during electroplating (El Ghawabi *et al.*, 1975). Symptoms persisted in 50% of the dyspneic workers in a 10-month nonexposure follow up period. These cyanide levels were associated with headache, weakness, giddiness, altered taste and smell, throat irritation, vomiting, lacrimation, thyroid enlargement and hematological disorders. Thyroid enlargement to a mild or moderate degree was found in 20 workers, although there was no correlation between the duration of exposure with either the incidence or the degree of enlargement. Increased blood hemoglobin and lymphocyte counts were present in the exposed workers. Additionally, punctate basophilia were found in 78% (28/36) of the exposed subjects (El Ghawabi *et al.*, 1975).

Another retrospective study of 36 former silver-reclaiming workers (Blanc *et al.*, 1985) with long-term exposure to hydrogen cyanide fumes found significant trends between the incidence of self-reported CNS symptoms during active employment (headache, dizziness, nausea, and bitter almond taste), the symptoms reported post-exposure, and a qualitative index of exposure retroactively defined by the investigators as low-, moderate-, or high-exposure through work histories. Some symptoms persisted for 7 months or more after exposure. None of the workers had palpable thyroid gland abnormalities, but clinical tests revealed decreases in vitamin B12 absorption and folate levels and statistically significant increases in thyroid-stimulating hormone levels, which in combination with the CNS effects, suggest long-term adverse effects associated with cyanide exposure.

V. Effects of Animal Exposures

There is little animal data for chronic inhalation exposure to hydrogen cyanide only two subchronic studies were noted by U.S. EPA, one in rabbits (Hugod, 1979, 1981) and the other in dogs (Valade, 1952). Continuous exposure of rabbits to 0.5 ppm HCN (0.55 mg/m^3) for either 1 or 4 weeks produced no microscopically detectable morphological changes of the lungs, pulmonary arteries, coronary arteries or aorta. This study observed a subacute inhalation NOAEL for HCN in rabbits of 0.5 ppm (Hugod, 1979, 1981). Four dogs exposed to 50 mg/m^3 hydrogen cyanide in a series of 30-minute inhalation periods conducted at 2-day intervals demonstrated extensive CNS toxicity, including dyspnea and vomiting, with vascular and cellular CNS lesions identified post-mortem (Valade, 1952).

No information was found regarding developmental and reproductive effects in humans for any route of hydrogen cyanide exposure. No animal studies utilizing inhalation or dermal exposure have been reported for either hydrogen cyanide or cyanide salts. Naturally occurring plant cyanogenic glycosides produce hydrogen cyanide when hydrolyzed. Dietary studies of the high cyanogenic glycoside cassava diet have shown adverse effects, increased runting and decreased ossification in hamsters (Frakes *et al.*, 1986), but not in rats fed cassava alone, or supplemented with potassium cyanide (Tewe and Maner, 1981). Hamsters with gestational cassava exposure did not display reproductive effects (Frakes *et al.*, 1986).

VI. Derivation of U.S. EPA RfC

Study	El Ghawabi *et al.* (1975), U.S. EPA (1995)
Study population	36 male electroplating workers
Exposure method	Discontinuous occupational inhalation exposures
Critical effects	CNS effects, thyroid enlargement, and hematological disorders
LOAEL	7.1 mg/m^3
NOAEL	Not observed
Exposure continuity	8 hr/day (10/20 m^3/day), 5 days/week
Average occupational exposure	2.5 mg/m^3 for LOAEL group
Human equivalent concentration	2.5 mg/m^3 for LOAEL group
Exposure duration	5 to 15 years
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Modifying factor	3 (lack of chronic and multi-generational reproduction studies)
Cumulative uncertainty factor	1,000
Inhalation reference exposure level	0.003 ppm (3 ppb, 0.003 mg/m^3, 3 $\mu g/m^3$)

U.S. EPA used a 3-fold subchronic uncertainty factor because the exposures continued over a significant fraction of an average human lifetime (>20% in some subjects).

The major strength of the RfC is the use of human health effects data. The major uncertainties are the lack of a NOAEL observation, the difficulty in estimating exposures, and the discontinuous and variable nature of the exposures.

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CHRONIC TOXICITY SUMMARY

HYDROGEN SULFIDE

(sulfur hydride; sulfuretted hydrogen; H_2S)

CAS registry number: 7783-06-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.9 μg/m^3 (**U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
Critical effect(s)	Nasal histological changes in B6C3F1 mice
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (AIHA, 1991)

Molecular formula	H_2S
Molecular weight	34.08
Description	Colorless gas
Specific gravity	1.189 @ 15° C (air = 1)
Boiling point	-60.7° C
Vapor pressure	1 atmosphere @ -60.4° C
Solubility	Soluble in water, hydrocarbon solvents, ether, and ethanol
Conversion factor	1 ppm = 1.4 mg/m^3 @ 25° C

III. Major Uses or Sources

Hydrogen sulfide (H_2S) is used as a reagent and an intermediate in the preparation of other reduced sulfur compounds. It is also a by-product of desulfurization processes in the oil and gas industries and rayon production, sewage treatment, and leather tanning (Ammann, 1986).

IV. Effects of Human Exposure

Although numerous case studies of acutely toxic effects of H_2S exist, there is inadequate occupational or epidemiological information for specific chronic effects in humans exposed to H_2S. No data on human reproductive or developmental effects from H_2S exposure were found.

Bhambhani and Singh (1991) showed that 16 healthy subjects exposed for short durations to 5 ppm (7 mg/m^3) H_2S under conditions of moderate exercise exhibited impaired lactate and oxygen uptake in the blood. Bhambhani and Singh (1985), reported that exposure of 42 individuals to 2.5 to 5 ppm (3.5 to 7 mg/m^3) H_2S caused coughing and throat irritation after 15 minutes.

In another study, ten asthmatic volunteer subjects were exposed to 2 ppm H_2S for 30 minutes and pulmonary function was tested (Jappinen *et al.*, 1990). All subjects reported detecting "very unpleasant" odor but "rapidly became accustomed to it." Three subjects reported headache following exposure. No significant changes in mean FVC or FEV_1 were reported. Although individual values for specific airway resistance (SR_{aw}) were not reported, the difference following exposure ranged from –5.95% to +137.78%. The decrease in specific airway conductance, SG_{aw}, ranged from -57.7% to +28.9%. The increase in mean SR_{aw} and decrease in mean SG_{aw} were not statistically significant.

V. Effects of Animal Exposure

Rats (Fischer and Sprague-Dawley, 15 per group) were exposed to 0, 10.1, 30.5, or 80 ppm (0, 14.1, 42.7, or 112 mg/m^3, respectively) H_2S for 6 hours/day, 5 days/week for 90 days (CIIT, 1983a,b). Measurements of neurological and hematological function revealed no abnormalities due to H_2S exposure. A histological examination of the nasal turbinates also revealed no significant exposure-related changes. A significant decrease in body weight was observed in both strains of rats exposed to 80 ppm (112 mg/m^3).

In a companion study, the Chemical Industry Institute of Toxicology conducted a 90-day inhalation study in mice (10 or 12 mice per group) exposed to 0, 10.1, 30.5, or 80 ppm (0, 14.1, 42.7, or 112 mg/m^3, respectively) H_2S for 6 hours/day, 5 days/week (CIIT, 1983c). Neurological function was measured by tests for posture, gait, facial muscle tone, and reflexes. Opthalmological and hematological examinations were also performed, and a detailed necropsy was included at the end of the experiment. The only exposure-related histological lesion was inflammation of the nasal mucosa of the anterior segment of the noses of mice exposed to 80 ppm (112 mg/m^3) H_2S. Weight loss was also observed in the mice exposed to 80 ppm. Neurological and hematological tests revealed no abnormalities. The 30.5 ppm (42.5 mg/m^3) level was considered the NOAEL for histological changes in the nasal mucosa. Adjustments were made by U. S. EPA to this value to reach the final RfC of 0.9 μg/m^3 as described below.

Male rats were exposed to 0, 10, 200, or 400 ppm H_2S for 4 hours (Lopez *et al.*, 1987). Samples of bronchoalveolar and nasal lavage fluid contained increased inflammatory cells, protein, and lactate dehydrogenase in rats treated with 400 ppm. Lopez and associates later showed that exposure to 83 ppm (116 mg/m^3) for 4 hours resulted in mild perivascular edema (Lopez *et al.*, 1988).

A study by Saillenfait *et al.* (1989) investigated the developmental toxicity of H_2S in rats exposed 6 hours/day on days 6 through 20 of gestation to concentrations up to 150 ppm (210 mg/m^3). No developmental defects were observed at any concentration of H_2S. However, maternal weight gain was depressed at 150 ppm (210 mg/m^3). No maternal effects were noted at 100 ppm (140 mg/m^3).

VI. Derivation of U.S. EPA RfC

Study	U.S. EPA, 1994; CIIT, 1983c
Study population	B6C3F1 Mice (10-12 per group)
Exposure method	Discontinuous inhalation
Critical effects	Histopathological inflammatory changes in the nasal mucosa
LOAEL	80 ppm (112 mg/m^3)
NOAEL	30.5 ppm (42.5 mg/m^3)
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	90 days
Average experimental exposure	5.4 ppm for NOAEL group
Human equivalent concentration	0.66 ppm (gas with extrathoracic respiratory effects, RGDR = 0.12, based MV = 0.04 m^3, SA(ET) = 2.9 cm^2)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factor	3 (lack of reproductive and developmental toxicity data)
Cumulative uncertainty factor	1,000
Inhalation reference exposure level	0.7 ppb (0.9 μg/m^3)

The adverse effects reported in chronic animal studies occur at higher concentrations than effects seen in acute human exposures. For example, human irritation was reported at concentrations of 2.5-5 ppm for 15 minutes (Bhambhani and Singh, 1985), yet no effects on laboratory animals were observed at concentrations up to 80 ppm for 90 days. This suggests either that humans are more sensitive to H_2S, or that the measurements in laboratory animals are too crude to detect subtle measures of irritation. However, the uncertainty factor and HEC incorporated by U.S. EPA attempts to account for these interspecies differences and database deficiencies.

The strengths of the inhalation REL include the availability of controlled exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathogical analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate long-term human exposure data and the lack of reproductive and developmental toxicity studies.

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